IS DISEASE AN IMPORTANT FACTOR IN THE DECLINE OF THE HOUSE SPARROW (*Passer domesticus*) IN GREATER LONDON?

' Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor in Philosophy by Daria Dadam '

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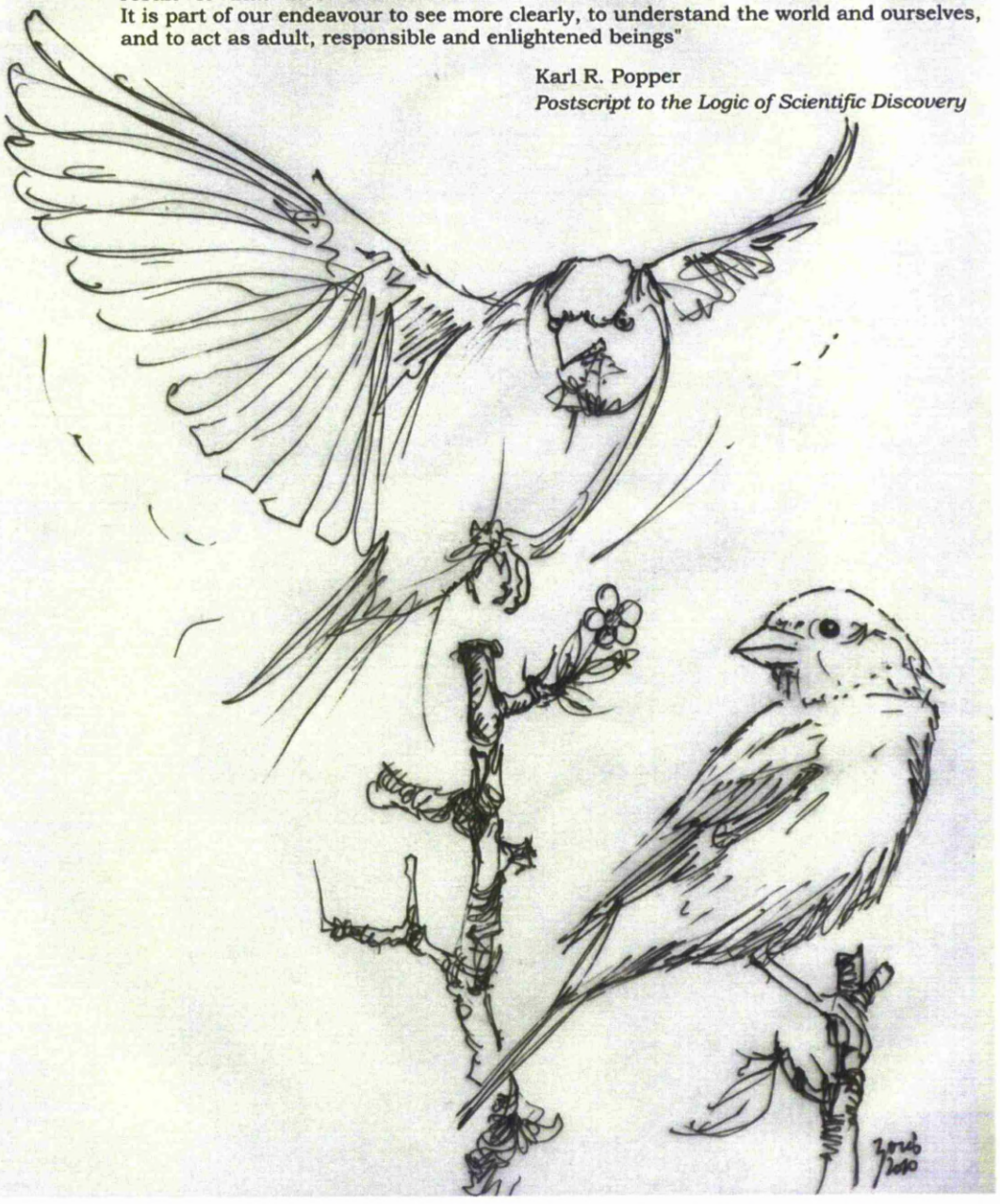
"Admittedly we can give some reasons for proposing a hypotesis, and for submitting it to critical discussion. But these are not justificatory reasons but are more in the nature of explanations of why - in the light of our aims (..) - we offer one theory rather than another."

"Science is not only, like art and literature, an adventure of the human spirit, but it is among the creative arts perhaps the most human: full of human failings and shorsightedness, it shows those flashes of insight which opens our eyes to the wonders of the world and the human spirit. But this is not all. Science is the direct result of that most human of all human endeavours - to liberate ourserves. It is part of our endeavour to see more clearly, to understand the world and ourselves, and to act as adult, responsible and enlightened beings"

Karl R. Popper
Postscript to the Logic of Scientific Discovery



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ABSTRACT

Daria Dadam – Is disease an important factor in the decline of the house sparrow (*Passer domesticus*) in Greater London?

The house sparrow is a small passerine bird that can be found in urban, suburban and rural environments in close proximity to human settlements. House sparrows used to be very common and widespread in the 19th and 20th centuries. The decline of this species started in rural areas in the 1970s, followed by the decline in urban populations from the 1980s. Since then, house sparrows have declined nationally in the UK by 66%, and by 68% in London between 1994 and 2009.

Several causes for the decline have been suggested, including lack of nest holes, increased predation by cats and sparrowhawks, pollution from unleaded vehicles, and lack of invertebrate food for chicks. A remaining hypothesis for the decline of the house sparrow in Great Britain, and one which had not yet been tested, was that this is due to infectious diseases. This is the hypothesis tested in this thesis.

The aim of this study were:

1. To investigate the prevalence, and where possible the intensity, of targeted infectious agents and parasites at the various sites.
2. To investigate markers – in particular haematological markers – of infectious diseases.
3. To investigate variation of infectious agents and parasites across years and seasons, and between age, sex, condition, breeding status, and moult status of infected birds.
4. To compare differences in prevalence and intensity of targeted infectious agents and parasites between: sites that were declining or non-declining, and sites that were provided with supplementary mealworms or not supplemented.
5. To investigate survival of house sparrows in relation to their prevalence and intensity of infectious agents and parasites.

There were three main parasite genera found in this study: *Plasmodium*, *Atoxoplasma*, and *Isospora*. Haematological values associated with prevalence and intensity of these parasites suggested that they were causing disease in their host. The three genera showed seasonal variation in prevalence and intensity, although *Plasmodium* prevalence was unexpectedly higher in winter. Adults had higher prevalence but lower intensity of infection than juveniles, and females had, overall, higher prevalence and intensity than males, although the results varied between parasite genera. Intensity of *Plasmodium* infection was negatively correlated with body condition, and prevalence and intensity were higher in breeding birds and in moulting individuals.

Plasmodium spp. intensity was higher in declining than in non-declining sites, as was prevalence of *Atoxoplasma* spp. . Prevalences of *Isospora* spp and *Atoxoplasma* spp. were higher in sites provided with supplementary mealworm feeders, but no association was found between this feeding experiment and *Plasmodium* spp.

Plasmodium spp. intensity of infection was negatively correlated with survival to the end of winter, but not with survival in the beginning of the winter period. Survival over the winter period was positively correlated with prevalence of *Plasmodium* spp., and negatively correlated prevalence of *Atoxoplasma* spp. .

This study has shown that parasites found in house sparrows in the London sites are causing disease in their host. They also varied across seasons and they showed an association with different aspects of the host's ecology. Intensity of *Plasmodium* spp, the most pathogenic of the three parasite genera found in this study, was higher in declining sites in London, and it was correlated with lower overwinter survival. Diseases act synergistically with other factors, such as predation, food scarcity, competition with other species and other stress factors to cause a detrimental effect in their host. The decline of the house sparrow in London is probably due to the synergistic effect of several factors, of which disease is likely to be an important one.

Chapter 1

INTRODUCTION

1. INTRODUCTION

1.1 THE HOUSE SPARROW

1.1.1 Biology

Description

The house sparrow *Passer domesticus* is a small passerine bird belonging to the genus *Passer* (Sibley and Monroe, 1990). The species is sexually dimorphic: the adult male has a chestnut brown upperside with white scapular *finestrae*, the head is white and brown with an ash-grey crown and it has a black mask around the eye. The chest is buff-colour with a black bib, which is more evident during the breeding season and which has been shown to increase in size with the age of the bird (Veiga, 1993; Solberg and Ringsby, 1997; Griffith *et* al., 1999). The function of the male's black bib might indicate the fitness of the individual, but this is still unknown and under debate (Anderson, 2006). The female is mainly brown on the upperparts and more buff than the male on the underparts. The sexes also vary in size, with adult males generally having a longer wing length than females (75-80 mm compared to 74-76 mm in females) (Svensson, 1992). House sparrows are highly sedentary and after natal dispersal they do not change colony and they rarely move more than 1-2 km from the breeding area (Summer-Smith, 1988).

Breeding

The house sparrow can be found in urban, suburban and rural environments (Summers-Smith, 1963). It is a granivorous bird but the chicks are dependent on invertebrates while in the nest. They are gregarious birds; they forage, roost and breed communally (Summers-Smith, 1963; Summers-Smith, 1988). Flock size varies greatly from a few birds to hundreds of individuals (Summers-Smith, 1954).

The house sparrow is both an open- and cavity nesting species (Summers-Smith, 1988). The pair builds a nest in a crevice of a house, a hole in a tree or nest box. It can also build a domed nest in the branches of trees and shrubs. The nest cup is lined with

feathers and hair or soft material, and sometimes some fresh green material is added (Heij, 1986). The nest site is defended by males and females against intraspecific attacks, often from birds of the same sex (Summers-Smith, 1958). The clutch size usually ranges from 3 to 6 eggs (Seel, 1968). Each egg usually is laid soon after sunrise (Anderson, 1997; Seel, 1968). First year females tend to lay smaller clutch sizes than older females (Anderson, 2006). The breeding season in the UK spans between April and August, and pairs have up to four clutches a year (Summers-Smith, 1988).

The incubation of eggs is performed almost exclusively by the female. The male lacks a brood patch, so it is thought that his role in incubation is limited to preventing the eggs from cooling too much in the absence of the female when she leaves the nest to feed (Anderson, 2006). In the female, the formation of the brood patch is controlled by a complex interaction of sex hormones which causes de-feathering of the breast and belly region and an increased vascularisation of the same area (Selander and Yang, 1966). The incubation period is variable, but usually is between 11 and 12 days. The eggs hatch asynchronously (Summers-Smith, 1988) in the order they were laid (Veiga, 1990). Egg failure can be due to a series of factors, including infertility, chilling, microbial infection, predation or desertion (Pinowski *et al.*, 1994, Kozłowski, 1991). Fledging success does not seem to vary greatly across European Countries and the success rate ranges from 42-84% (Anderson, 2006). The main recorded causes of nestling failure are predation, starvation, desertion, and being pushed out of the nest (Anderson, 2006).

Moult and feathers

When the breeding season is over (in August in the UK), both adults and juveniles undergo a complete moult, a process that takes about 60 days in adults and 80 days in juveniles (Ginn and Melville, 1983). By autumn, once the moult is complete, all individuals of each sex are morphologically similar, making discerning older and first-year birds by plumage impossible (Svensson, 1992). Moult is an important part in a bird's life-cycle because it is time and energy consuming (Murphy and King, 1992), but maintaining feathers in good condition is essential for efficient flight and insulation

(Anderson, 2006). The birds keep their plumage in good condition throughout the year by preening, bathing and dust-bathing to control ectoparasites (Polani, 2000).

Feeding

The house sparrow is an opportunistic forager and feeds on insects as well as seeds and other available suitable food (Gavett and Wakely, 1986; Summers-Smith, 1963) (at ZSL London Zoo the sparrows are frequently seen eating meat that is given to vultures). They are highly sociable birds and they feed in flocks, which gives them a higher protection against predators than if they were solitary (Pulliam *et al.*, 1982; Pulliam, 1973). The peak of foraging activity in winter is early in the morning, with a second, lower, peak shortly before sunset (Beer, 1961). In summer, sparrows can arrive at feeding stations before dawn (Lima, 1987). Large flocks are usually reported in rural settings and they contain many juveniles in summer (Anderson, 2006). Winter flock sizes tend to be smaller, containing 5-15 birds (Beer, 1961). In the UK, the house sparrow is the most common species at garden feeding stations (Cowie and Hinsley, 1988), particularly in autumn, and the number of birds visiting feeding stations in winter is inversely correlated with ambient temperatures (Breitwisch and Hudak, 1989).

Individuals in a flock assume different foraging roles. Some individuals, termed "searchers" (Barnard and Sibly, 1981), find food without interaction with other birds, while other birds, termed "copiers", observe and learn from other birds about food location. House sparrows learn to eat a new food item through social learning by observing other individuals, with juveniles most prone to this behaviour (Turner, 1965). When sparrows arrive at a new site they try to attract other conspecifics before starting to forage, especially when the risk of predation is perceived to be high (Newman and Caraco, 1989). The first sparrow to arrive on site, the "pioneer", usually calls to attract other sparrows (Elgar, 1986).

Adult sparrows are mainly granivorous. The diet of urban individuals is quite different from that of rural birds. The latter consume grains and insects, while the former feed mainly on seeds coming from bird tables, insects and other types of seeds, like weeds

(Gavett and Wakeley, 1986). The diet of egg-laying females is richer in animal protein than at other times of their life cycle (Pinowska, 1975). Juvenile birds beg parents and take invertebrates up to 25-30 days of age, when already out of the nest (Mueller, 1986). Mueller (1986) suggested that the sudden switch to granivorous diet occurs suddenly, and it may represent an innate behaviour.

1.1.2 The decline

The house sparrow used to be a ubiquitous bird in habitats associated with human beings, in urban, suburban and agricultural settings. The species was so common and widespread that it was considered a pest in the 19th and 20th centuries, and eradication programmes were introduced (Summers-Smith, 1963).

In Europe the overall population was stable between 1970-1990, after which the species started to decline (BirdLife International, 2004). Data on sizes of house sparrow populations in Europe are not available before the 1970s, because many countries did not have ornithological surveys (De-Laet and Summers-Smith, 2007), and in those that had, the species was largely overlooked during bird counts (Freeman and Crick, 2002). Countries reporting house sparrow population declines include the UK, Germany, France, Italy, the Netherlands, Belgium, Norway, Sweden, Finland, Latvia, Estonia, Lithuania, the Czech Republic, Slovakia, Croatia, Ukraine, Greece and Turkey (BirdLife International, 2004).

These declines have occurred irrespective of habitat type, with sparrow populations affected in both the urban/suburban garden habitat and the farmland landscape (De-Laet and Summers-Smith, 2007). Reasons suggested for the decline in farmland areas include lack of food as a result of changes in farmland practice, such as a shift from spring to autumn sowing, better grain storage facilities that reduce opportunistic feeding by sparrows, especially in winter, and the widespread use of herbicides and insecticides which resulted in decreased food availability in winter and during the reproductive

season (Chamberlain *et al.*, 1999; Robinson and Sutherland, 2002), leading to local extinctions (Hole *et al.*, 2002).

In the UK, house sparrow populations have declined by almost 60% since the 1970s, initially in the wider countryside and later in gardens (Robinson *et al.*, 2005). Historical data from the BTO's Common Bird Census and Garden Bird Feeding Survey, and the recent data from the BTO/JNCC/RSPB Breeding Bird Survey, showed that decline in farmland populations started in the 1970s, while in urban environments the decline started around 1983, and since then populations in rural gardens have declined by 48% and in urban garden by 60% (Robinson *et al.*, 2005). The decline, in urban, suburban, and farmland areas is not entirely understood and a single indisputable cause has not yet been found, although several hypotheses have been proposed.

London decline

The decline of the urban house sparrow is not evenly distributed throughout the British Isles: the decline has been marked in London, Edinburgh, Dublin, Bristol, Norwich, and Glasgow (Sanderson, 1996; Dott and Brown, 2002; Prowse, 2002; Bland, 1998; Paston, 2000; Summers-Smith, 1999), but other cities, such as Manchester and some urban populations in Wales and South West England have not reported a negative population trend (Robinson *et al.*, 2005). The London house sparrow population declined by 68% during the period 1994-2009 (Risely *et al.*, 2010), although the peak decline in urban environments elsewhere in Great Britain occurred in the period 1983-1994 (Robinson *et al.*, 2005). The house sparrow population decline in London was first reported by Sanderson (1996), who noticed a huge decline in house sparrow numbers in Kensington Gardens. Four years later, the decline of the house sparrow in London and elsewhere had become so evident that The Independent newspaper put up a reward of £5,000 for the first scientific study that could explain the decline of this species (McCarthy, 2000).

Several hypotheses have been suggested for the decline of the urban house sparrow. One posits that a lack of nest sites due to new and renovated buildings providing fewer crevices where the species can nest (Noble and Eaton, 2002). Other hole-nesting species,

sympatric to the house sparrow in London, however, have not declined (Risely, 2010; Hewlett, 2002). House sparrows can also be an open-nest species (Summers-Smith, 1988), and in London they have not used nest-boxes provided by the RSPB installed during a campaign to provide nesting habitat for them. This may indicate that, at least in those colonies, a lack of nest sites was not an important factor, although no data on nest cavities or open-nests are available from the study sites.

A second hypothesis states that the decline is due to increased predation pressure from cats (*Felis catus*) (Beckerman *et al.*, 2007; Backer *et al.*, 2005), and sparrowhawks (*Accipiter nisus*) (Bell *et al.*, 2010; MacLeod *et al.*, 2006), along with increased competition by sympatric species, such as pigeons (*Columba livia* and *Columba palumbus*) (Bland, 1998). In support of this hypothesis, Beckerman *et al.* (2007) used theoretical models to estimate the impact of cat predation and extrapolated from observational data collected from questionnaire distributes to cat owners (Baker *et al.*, 2005; Woods *et al.*, 2003). A field study by the RSPB on cat predation in London, however, showed that this was not significant in the decline of the house sparrow (Mallord *et al.*, 2010), while a relationship has been shown between the house sparrow decline and the presence of sparrowhawks in the UK on a correlational basis (Bell *et al.*, 2010), but no causal link has been found.

A further hypothesis states that the advent of lead-free petrol in the 1990s led to the release of harmful substances (Summers-Smith, 2007), but other urban species that likely would have been affected by the same problem have not declined. Another hypothesis links food scarcity to population decline through reduced survival of nestlings (Peach *et al.*, 2008). This idea was based on the results of a study in Leicester which showed that house sparrow chicks fed on a protein-poor diet had a lower fledging success and body mass (Peach *et al.*, 2008; Vincent, 2005). Lack of protein-rich food can cause starvation and also can weaken the immune system (Apanius, 1998). A lack of invertebrate prey, therefore, could cause a decline, especially in a species that is multi-brooded. The same negative effect might not be evident in other species that feed their nestlings on a diet based on invertebrates, but that have only one brood a year.

Another recent hypothesis pointed at mobile phone masts as culprits of the decline (Balmori, 2009; Balmori and Hallberg, 2007) following circumstantial evidence of an association between the decline of house sparrows in a park in Spain and the building of three masts. This hypothesis, however, continues to lack evidence of a causal relationship and it has been dismissed by the physics scientific community (e.g. by Alan Preece, Professor of Medical Physics in a comment reported by Townsend (2003)).

A remaining hypothesis for the decline of the house sparrow in Great Britain, and one which has not yet been tested, is that this is due to infectious disease. It is this hypothesis that I test in this thesis.

1.2 PATHOGENS

Pathogens are organisms (bacteria, virus, fungi and parasites) that are capable of causing disease (Pearsall, 1998), which can be defined as any departure from health that produces specific signs or symptoms (Pearsall, 1998), on a physiological, physical, reproductive or behavioural level (Friend *et al.*, 2001). Pathogens have the ability to control animal populations by modifying the host's chances of survival and/or of its reproductive success (Anderson and May, 1979; May and Anderson, 1979) and, thus, they can impose a strong selection pressure on their hosts (reviews in Møller, 1997).

Parasites are organisms that live in or on a host, from which they derive nutrients (Clayton and Moore, 1997). Parasites can be pathogens if they have the potential to cause disease (Clayton and Moore, 1997). There are costs associated with parasitism, which include: loss of resources that the parasite extracts directly from the host (e.g. blood), competition between the parasite and the host for resources (e.g. nutrients), cost incurred by the host for defence against the parasite (including grooming, avoidance of some environments, behavioural adaptations, cost of developing and activating an

immune response), and costs of injuries to tissues affected by the parasite (Wobeser, 2008).

The most obvious costs usually are associated with competition for resources and energy, which are limited and have to be allocated between different physiological activities (Woebeser, 2008; Sheldon and Verhurst, 1996), hence the idea that there may be a trade-off between the energy allocated for fighting, and interacting with, parasites and that used for other physiological activities (Sheldon and Verhurst, 1996). For example, the cost of producing an antibody response to a novel antigen in a female house sparrow has been estimated to be equivalent to the energy needed to produce half an egg (Martin *et* al., 2003). Similarly, the energy utilised for physiological activities, such as breeding, may reduce the host's ability to mount an immune response (Nordling *et al.*, 1998; Siikamäki *et* al., 1997; Deerenberg *et al.*, 1997; Oppliger *et al.*, 1996; Ots and Hõrak, 1996; Richner *et al.*, 1995; Norris *et al.*, 1994). The trade-off between competing energy needs is not constant, but changes according to the host's physiological status (e.g. age, breeding status) and to environmental factors, such as weather, and parasite abundance (Woebeser, 2008).

The trade-off between the effects of the parasite on the host and the host's normal physiological demands is thought to have resulted in the evolution of sexually-selected traits that provide honest , i.e. costly (Zahavi, 1977), signals of an individual's quality and fitness; only the fittest individuals can express those sexually selected characters and fight infections at the same time (Hamilton and Zuk, 1982). Plumage brightness (Hamilton and Zuk, 1982) in several species, or tail length in barn swallows (*Hirundo rustica*) (Møller , 1991; Møller, 1990) are thought to be examples of parasite-mediated sexually-selected traits.

Social species, such as the house sparrow, may incur higher costs due to parasitism than more-solitary species, because sociality is associated with increased risks of parasitism (Møller *et al.*, 2001; Côte' and Poulin, 1995; Moller *et al.*, 1993; Davies *et al.*, 1991), increased social stress (Gross and Siegel, 1983), and reduced immunity (Apanius, 1998).

Thus, it has been suggested that social animals require a greater investment in their immune system than non-social species (Møller and Erritzøe, 1996).

The cost of parasitism is not always obvious. Apparently neutral effects of parasites may be due to (Woebeser, 2008):

a) an effectively trivial cost to the host resulting in a not-detectible effect;

b) the cost is present but tolerable, specifically in those individuals that have enough energy to afford losing resources to the parasites without incurring significant negative effects on other functions (but this equilibrium might be altered with changing environmental circumstances);

c) the cost of parasitism is masked by other factors, such as predation – predators may take the easiest to catch (weakest) individuals (Temple, 1987), or parasites might make some individuals more prone to predation, as shown in the example of the effects of the intestinal nematode, *Trichostrongylus tenuis* on the red grouse (*Lagopus lagopus scotica*) (Hudson *et al.,* 1992a). Navarro *et al.* (2004), however, showed that house sparrows exposed to potential predators (cats) had a reduced immune response and higher prevalence and intensity of *Haemoproteus* spp. infection than individuals exposed to non-predatory species. The impacts of exposure to parasites and to predators can, therefore, be difficult to tease apart and might be additive;

d) the cost of parasitism is substantial but the individuals are examined/sampled at the wrong time of the year, or at the wrong life stage, or the cost of parasitism could be paid later in life. Møller (1994) suggested that parasitized nestlings of barn swallows might pay the cost of parasitism with reduced survival later in life, or the parents might pay the cost through reduced survival or future breeding success due to the extra energy required to fledge parasitized young. In an experiment of antiparasite treatments on female Eider ducks (*Somateria mollissima*) Hanssen and colleagues (Hanssen *et al.*, 2003) showed that, among females that had bred successfully, there was no effect of the treatment. Among individuals that had failed to breed, however, untreated birds had a higher mortality rate than those treated for parasites (Hanssen *et al.*, 2003).

The effect of parasites on population dynamics is very difficult to investigate, not least owing to the reasons listed above (a-d). However, some studies have shown a link between parasites and the population dynamics of their hosts. For example, Hudson *et al.* (1992b) found that the nematode *Tricostrongylus tenuis* had a regulatory effect on a red grouse population via decreased breeding success and winter survival. Van Riper III *et al.* (1986) highlighted the role of *Plasmodium* spp. as a regulatory agent of populations of Hawaiian native birds. On the North American continent, Hochachka and Dhondt (2000) showed a causal relationship between *Mycoplasma gallisepticum* infection, causing mycoplasmal conjunctivitis, and the decline of the house finch (*Carpodacus mexicanus*) in eastern USA, and LaDeau *et al.* (2007) identified West Nile Virus as a cause of decline of some North American bird species. In the UK, the decline of the greenfinch (*Carduelis chloris*) has recently been shown to be a consequence of infection with the protozoan *Trichomonas gallinae* (Robinson and Lawson *et al.*, 2010).

Wild birds are natural reservoirs of bacteria, protozoa, helminth parasites and viruses. Garden birds are at particular risk of contracting diseases from one another through the common use of feeding stations where faeces and food are mixed, and the sharing of small enclosed water sources such as bird baths and ponds (Brittingham *et al.*, 1988). Pathogens cited as causing mortality in garden birds in Great Britain include bacteria, such as *Salmonella enterica* serotype Typhimurium (*S.* Typhimurium) (Tizard, 2004; Refsum *et al.*, 2003; Pennycott *et al.*, 1998; Kirkwood and Macgregor, 1997; Brittingham *et al.*, 1988; Tizard *et al.*, 1979; Locke *et al.*, 1973), *Yersinia* spp (Kirkwood and Macgregor, 1997; Brittingham *et al.*, 1988) and *Escherichia coli* of different subtypes (Foster *et al.*, 2006; Pennycott *et al.*, 1998; Brittingham *et al.*, 1988); and protozoa, incuding *Trichomonas gallinae* (Robinson and Lawson et al. 2010), and coccidia (*Eimeria* spp., *Isospora* spp.) (Dolnik, 2006) . There are numerous other pathogens that can affect birds, including other bacteria, viruses, fungi, and protozoa (Atkinson *et al.*, 2008; Thomas *et al.*, 2007) that were beyond the scope of this project.

1.2.1 Bacteria

The bacteria most commonly cited as killing house sparrows are *Salmonella enterica* serotype Typhimurium, and *Escherichia coli* subtype 086 (Pennycott *et al.*, 1998).

Salmonella enterica serotype Typhimurium

Salmonella spp. primarily are enteric bacteria, and the faecal-to-oral route of exposure is the main route of transmission (Daoust and Prescott, 2007). There are almost 2,500 serotypes of *Salmonella* spp. Bacteria (Daoust and Prescott, 2007). Different serotypes adapt to different host species, some individuals of which can act as carriers. The disease, salmonellosis, can then emerge as outbreaks within the host population when conditions are favourable (Pennycott *et al.*, 1998). Such conditions involve interactions between host and the environment, including impaired immune system of the host (Daoust and Prescott, 2007).

Salmonella Typhimurium appears to have adapted to songbirds, including to the house sparrow (Pennycott *et al.*, 2010; Pennycott *et al.*, 2006; Pennycott *et al.*, 2002; Daoust *et al.*, 2000) causing sporadic epizootics both in North America and in Europe. Outbreaks of salmonellosis in North America have involved pine siskins (*Carduelis pinus*) in North-Eastern USA in 1988; pine siskins, evening grosbeaks (*Coccothraustes vespertinus*), purple finches (*Carpodacus purpureus*), and house sparrows in 1992-1993 in Western North America; and finches (*Carduelis* spp.) including redpolls (*Carduelis flammea*) in 1997-1998 in the Eastern and Midwest areas of USA and Atlantic Canada (reported in Daoust *et al.*, 2000). In Europe, salmonellosis outbreaks have been recorded in Norway, involving over 400 passerines from 1969 to 2000 and affecting mainly bullfinches (*Pyrrhula pyrrhula*), greenfinches, siskins (*Carduelis spinus*), common redpoll, and house sparrow (Refsum *et al.*, 2002). In Great Britain, deaths caused by *S.* Typhimurium infections have been reported since the 1960s (Tizard, 2004), involving finches and house sparrows (Wilson and McDonald, 1967). In the 1990s, salmonellosis outbreaks were reported as an almost-annual occurrence with a winter seasonality (Pennycott *et* al., 2010; Pennycott *et al.*, 1998).

Birds with salmonellosis show non-specific clinical signs, which include fluffed–out feathers, rapid breathing, shivering, weakness, lethargy and apparent indifference to their surroundings. Death in infected birds usually occurs within 24 h of the onset of disease (Daoust and Prescott, 2007). Young birds appear to be particularly susceptible to infection (Hall and Saito, 2008; Daoust and Prescott, 2007; Butterfield *et al.*, 1983). Explanations for the discrepancy in age-dependent susceptibility included under-development, in young birds, of the natural intestinal flora, which has an inhibitory effect on *Salmonella* bacteria (Holt, 2000), and a lack of an acquired immunity to the bacterium in young birds (Benskin *et al.*, 2009).

The role of a carrier state in healthy birds in the epidemiology of salmonellosis of garden birds is unknown. In wild birds exposed to salmonellae the carrier phase seems to be short, generally 4 days (Girdwood *et al.* 1985), although this varies from species to species. *Salmonella* Typhimurium can, however, survive outside the host if the environmental conditions are favourable. For example, it can remain viable for up to nine months in soil at temperatures below 25 °C (Mair and Ross, 1960), and in bird food for at least 18 months at 11°C, 16 months at 25 °C, and 40 days at 38 °C (Williams and Benson, 1978), but survival of the bacterium is reduced with high moisture (Williams and Benson, 1978).

The feeding ecology of species is thought to be a key aspect in the acquisition of infection with *S.* Typhimurium (Monghan *et al.*, 1985). It has been suggested, for example, that the feeding behaviour of birds belonging to the family Paridae, in which individuals take one item at a time and fly away with it, rather than eating on the bird table or on the ground, protects them against exposure to faecal-borne bacteria; the Paridae are rarely affected by salmonellosis compared to other common garden passerines (Refsum *et al.*, 2003).

Feeding stations have been identified since the 1960s as a potential source of disease transmission amongst birds due to the high concentration of birds repeatedly feeding at the same site (Wilson and MacDonald, 1967; Locke, *et al.*, 1973; Brittingham and

Temple, 1986; Pennycott *et al.*, 1998). There is some evidence that the population density of birds at feeding stations is a driver of salmonellosis outbreaks (Kirkwood, 1998). Ground-feeding species, such as house sparrows, are thought to be at a greater risk of infection at feeding stations than birds that feed from hanging feeders because of increased faecal contamination of food on the ground below feeders (Tizard, 2004). Also, it has been postulated that competition at feeding stations might increase stress levels and hence increase disease susceptibility (Daoust and Prescott, 2007). Pennycott (2002) stressed that some species that routinely use feeding stations, such as house sparrows, have been declining dramatically in recent decades and he suggested that disease contracted at bird feeders might play a role in these declines.

Escherichia coli

Escherichia coli causes fewer cases of disease in garden birds than *Salmonella* spp. (Pennycott *et al.*, 1998). However, there are some documented cases of mortality due to this bacterium in Scotland (Foster *et al.*, 2006; Pennycott *et* al., 2002; Pennycott *et al.* 1998) and in America (Brittingham, *et al.*, 1988). Nestling house sparrows can also be affected: a Polish study suggested a 20.4% incidence of *E. coli* infection in unhatched eggs, 57.1% in dead nestlings, and 25.6% in live chicks (Pawiak *et al.*, 1991). Nestlings from which *E. coli* was isolated had a significantly lower body mass than chicks that were not infected, and the bacterium was isolated in 78% of dead chicks (Kozłowski *et al.*, 1991) – however, *E. coli* is a normal part of the healthy gut flora of many species, and can spread through the body *post mortem*.

Transmission between individuals occurs through the faecal-oral route, and bird feeders and bird tables are considered the main vector of transmission (Pennycott *et al.*, 2005; Pennycott *et al.*, 1998).

1.2.2 Haemoprotozoa – Haemoproteus, Plasmodium and Leucocytozoon

Haemoprotozoa are protozoan (single-celled) eukaryotic organisms that parasitise their host's blood cells for at least one stage of their life-cycle. They are transmitted by vectors such as biting flies. The haemoprotozoan species belonging to the genera

Haemoproteus, Plasmodium and Leucocytozoon are considered the most likely to be pathogenic to passerine birds (Atkinson and Van Riper III, 1991). In contrast, helminth haemoparasites, such as microfilariae, generally are considered non-pathogenic in passerines (Campbell and Ellis, 2007).

Haemoproteus

Haemoproteus spp. are among the most widespread of the haemoprotozoa, affecting 72 families of birds world-wide (Valkiūnas, 2005), with the greatest number of susceptible species within the order Passeriforms (Bennett, 1993). Some *Haemoproteus* spp. can be highly pathogenic in wild birds, including house sparrows (Paperna and Gill, 2003), but their role as disease agents in populations of wild birds is still mostly unknown (Atkinson, 2008a).

Vectors of *Haemoproteus* spp include flies belonging to the genus *Culicoides* and ectoparasitic hippoboscid flies belonging to the genus *Icosta*. The life-cycle involves sexual (gametogenesis) and asexual (sporogony) reproduction in the vector, as well as asexual reproduction (merogony) in the avian host. The sexual cycle occurs in the vector after it has taken a blood meal containing female macrogametocytes and male microgametocytes, which undergo fertilisation in the midgut of the host and produce motile zygotes. These undergo an asexual sporogonic cycle development in another section of the vector midgut and become spherical oocysts. Each oocyst contains multiple sporoblasts, each of which produces thousands of sporozoites. Sporozoites released from the oocysts in the gut of the vector enter the haemocoel of the insect, eventually invading the salivary glands to be inoculated into the next vertebrate host at the following blood meal (Atkinson, 2008a). Sporozoites inoculated into the avian host develop within cells of the lymphoid-macrophage system, capillary endothelium and/or myofibroblasts, usually undergoing one or more pre-erythrocytic asexual cycles (merogony) before entering circulating erythrocytes (Atkinson, 1986), where they develop as gametocytes, becoming infective to vectors around 10 days after invading the blood cells (Atkinson, 2008). *Haemoproteus* spp erythrocytic gametocytes are

characterised by golden-brown or black pigment granules; they can be distinguished from *Plasmodium* spp. by the absence of meronts in the erythrocytes (Atkinson, 2008a).

Peak parasitaemia occurs in the peripheral blood about 21 days post-infection but falls rapidly to low intensities after around 7 days of high parasitaemia. A second, smaller, peak may occur around 35 days after initial infection (Atkinson, 1986). The intensity of the infection, after the initial acute phase, varies greatly between individual hosts and it depends on the complex interactions between host immune system, seasonal changes in photoperiod and associated hormonal changes during the breeding season (Atkinson, 2008a).

A seasonal increase in intensity of infection, referred to as "spring relapse", often occurs in temperate areas during the host's breeding season. A second peak can occur in late summer, when the number of vectors is at its peak, and the host population is inflated by recently fledged immunologically-naïve juveniles (Atkinson and van Riper, 1991; Valkiūnas *et al*, 2004). Experimental evidence has shown that photoperiodic physiological changes leading to increased levels of hormones, such as melatonin, also are important stimuli for a relapse of parasitaemia for some species of *Haemoproteus* (Valkiūnas *et al*, 2004).

Other factors that can affect intensity of infection include stress-mediated immunosuppression associated with reproduction (Siikamäki *et al.*, 1997), food availability (Appleby *et al.*, 1999), concurrent infection with other parasites (Cox, 1987), and exposure to predators (Navarro *et al.*, 2004). Other intrinsic factors associated with the prevalence of *Haemoproteus* include host specificity of the parasite (Bennett, 1993), immune competency (Ricklefs, 1992), host age and sex (Davidar and Morton, 1993; McCurdy *et al.*, 1998), migratory biology of the host species (Figuerola and Green, 2000; Smith *et al.*, 2004), and host foraging and nesting behaviour (Garvin and Remsen, 1997). Extrinsic factors, such as habitat, geographic region and season, are important because they can influence vector presence and abundance (Mendes *et al*, 2005; Sol *et al.*, 2000).

Clinical signs of *Haemoproteus* infection may not be evident at low infection levels, but become more apparent at high levels of parasitaemia (Atkinson 2008a). They include weakness, apathy, and anorexia (Atkinson, 2008; Cardona *et* al., 2002), Increased number of circulating lymphocytes, heterophils, basophils, eosinophils and monocytes, are also associated with infection, probably reflecting a response to the erythrocytic and pre-erythrocytic stages of the infection, particularly at the stage of invasion of the host erythrocytes (Garvin *et al.*, 2003; Ots and Hõrak, 1998). No changes in plasma protein concentration, haemoglobin concentration, packed cell volume, or weight were observed between infected and non-infected blue jays (*Cyanocitta cristata*) (Garvin *et al*, 2003). Whilst infection with *Haemoproteus* sp. did not cause anaemia in domestic turkeys (Atkinson *et al.*, 1988) or in great tits (Ots and Hõrak, 1998), regenerative anaemia was reported as a consequence of infection in snowy owls (*Nyctea scandiaca*) (Evans and Otter, 1998).

Other reported consequences of *Haemoproteus* sp. infection include detrimental effects on immunity, reproductive success and condition of infected individuals (Allander and Bennett, 1995; Ots and Hõrak, 1998; Merino *et al.*, 2000; Sanz *et al.*, 2001). Several studies have found infection to decrease survival rates of infected birds (Nordling *et al.*, 1998; Dawson and Bortolotti, 2000; Hõrak *et al.*, 2001; Sol *et al.*, 2000). Korpimäki *et al.* (1995) showed that female kestrels (*Falco tinnunculus*) laid smaller and later clutches when paired with parasitized males. Other studies, however, failed to find any association between *Haemoproteus* infection and survival (Davidar and Morton, 1993) or reproductive success (Dale *et al.*, 1996).

Wiehn and Korpimaki, (1998) showed a relationship between food abundance and intensity of parasitaemia, with the latter decreasing when food abundance was higher. Also, the intensity of parasitaemia was found to increase as the host's reproductive effort increased in the great tit (*Parus major*) (Norris *et al*, 1994; Ots and Hõrak, 1996; Allander, 1997) and in the pied flycatcher (*Ficedula hypoleuca*) (Siikamäki *et al*, 1997; Nordling *et al.*, 1998).

It is likely that the effect of *Haemoproteus* sp. infection on the host depends on several factors, including concurrent parasitism by other parasites, age and condition of the host, and whether the infection is acute of chronic (Atkinson, 2008a). Perhaps an overlooked factor, however, is the species (and virulence) of the parasite. Most studies identify *Haemoproteus* sp. infection via the examination of blood smears, so the species of parasite is not identified. It is possible that differences in outcomes of infection are consequent to the species of the *Haemoproteus* parasite involved and its adaptation to the host species under study. Although a range of consequences of *Haemoproteus* sp. infection have been shown at the level of the individual host, whether this parasite can have any impact on wild birds at the population level is unknown (Atkinson, 2008a).

Plasmodium

The genus *Plasmodium* contains more than 40 species of mosquito-trasmitted protozoan parasites that cause avian malaria. *Plasmodium* spp vary in host, vector, distribution and pathogenicity, and they are closely related to haemosporidian parasites of the genera *Haemoproteus* and *Leucocytozoon*, but they can be differentiated from both by the presence of asexual reproduction stages (merogony or schizogony) in circulating erythrocytes. (Atkinson, 2008b).

The life cycle of *Plasmodium* spp (described in Atkinson, 2008b) begins when the mosquito vector inoculates the infective sporozoites into a susceptible host (Huff and Coulston, 1944). In the initial stages the protozoan does not invade circulating erythrocytes, and it is therefore defined as pre-erythrocytic stages of infection. Sporozoites undergo a first asexual reproduction to create cryptozoites, which mature within 48h and subsequently invade cells of the lymphoid-macrophage system of the brain, spleen, liver, kidney and lung where they undergo a second asexual generation. These metacryptozoites mature and release morozoites that are capable of invading circulating erythrocytes, where they undergo asexual reproduction (merogony) and develop in 24-48 h into either mature meronts or gametocytes that are infective to mosquito vectors. Merozoites usually destroy the host's erythrocyte when released. Male gametocyte typically stain pink with Giemsa stain, and female gametocyte stain pale

blue. The parasite ingest host's haemoglobin while growing in the erythrocytes, and the pigment typical of malarian parasites is produced as a by-product of the ingestion of haemoglobin and may appear as black or golden-brown granules.

Merogony can continue to occur in the circulating erythrocytes throughout the host's life, and merozoites can re-invade organ tissues and continue development. Gametocytes do not undergo further development until they are in the midgut of a suitable mosquito vector, where they undergo gametogenesis to form gametes. The male gamete then fertilises the female one and within 24 h a motile zygote develops, which is capable of penetrating the midgut walls of the host and develop into a oocyst under the basal membrane of the mosquito's midgut. Oocysts undergo asexual reproduction (sporogony) to produce thousands of sporozoites, which are released in the hemocoel of the host once the oocysts are mature, usually within 7 days depending on ambient temperature. Sporozoites move through the hemocoel to the salivary glands and the salivary duct, accessing the new avian host when the mosquito takes its blood meal and passes saliva into the host.

The acute phase of infection in the avian host typically occurs after 6-12 days after the parasite penetrates in the blood of the host. The level of parasitaemia then decreases rapidly to chronic infection as the immune system of the host brings the infection under control, but chronic infection typically lasts for the lifetime of the bird due to recrudescing infections caused by circulating parasites and meronts found in the tissues (Bishop et al 1938).

The intensity of the chronic infection seems to depend on the complex interaction between the host immune system and hormonal changes associated with seasonal changes in photoperiod and the host's breeding season. An increase in prevalence of infected individuals occurs during the breeding season when previously infected individuals relapse, and a second peak may occur in late summer, when vectors and immunologically naïve juvenile birds are at their peak number (Atkinson and van Riper, 1991). This phenomenon is called the "spring relapse", and it may be due to changes in

melatonin (Valkiunas, *et al.*, 2004), or stress-induced high levels of corticosterone (Valkiunas, *et al.*, 2004; Applegate, 1970; Applegate and Beaudoin, 1970). Valkiūnas and colleagues (Valkiūnas *et al.*, 2004) noticed a relapse in haemoparasites in blackcaps (*Sylvia atricapilla*) associated with increased day length, and suggested that the relapse may be associated to changes in melatonin. In the same study the authors noticed that the relapse could also associated with exposure of the birds to environmental stressors, which cause a raise in corticosterone (Gross and Siegel, 1973), which in turn has been associated to relapses of parasitaemia, for example of *Plasmodium* in house sparrows (Applegate, 1970; Applegate and Beaudoin, 1970).

Corticosterone levels increase also as a result of social stress (Gross and Siegel, 1973), and circulating levels of testosterone increase in males as a result of aggressive interactions (Wingfield and Hahn, 1994). Applegate and Beaudoin (1970) have not found, however, a direct link between gonadotrophin hormones and relapse of *Plasmodium* spp. infection, and they concluded that the relapse was not associated with those hormones. During the breeding season a combination of stressors may contribute to the relapse of chronic infections, while the emergence of vector populations and immunologically-naïve juveniles contribute to the increase in prevalence noticed (Cosgrove *et al.*, 2008; Schrader *et al.*, 2003; Beaudoin *et al.*, 1971) including in house sparrows (Applegate, 1971). Other factors affecting the intensity of parasitemia included stress-mediated changes in the immune-system due to the effort of breeding (Siikamäki *et al.*, 1997), food availability (Appleby *et al.*, 1999), co-occurring infections with other parasites (Wright *et al.*, 2005), and even exposure to predators (Navarro *et al.*, 2004). Stress can lead to a reduction in physiological resources available for reproductive effort (Apanius, 1998).

Birds infected with *Plasmodium* spp. are typically anemic, lethargic, anorexic and have ruffled feathers (Atkinson, 2008b). Haematocrit value may fall by over 50%, but wild birds with acute infections are rarely captured with mist nets (Atkinson, 2008b). The intensity of the infection and related mortality depend on the number of infected sporozoites that the bird has been infected with (Atkinson *et al.*, 2000). Mortality

generally occurs at the peak of the infection, from day 5 to day 11 after infection (Williams, 2005). Birds infected with *Plasmodium* spp. show higher leucocyte count than non-infected individuals (Figuerola *et al.*, 1999), but no response is evident around pre-erythrocytic parasites, and the host seems unable to totally clear the infection (Atkinson 2008b). The persistence of sub-clinical infection may make birds more vulnerable to relapses of erythrocytic infections if the immunity of the host is compromised by infection with other parasites or stress, providing some evidence of the cost associated with mounting an immune response (Atkinson, 2008b).

The evidence of epizootic die-offs caused by *Plasmodium* spp. parasites in the wild is scarce (Atkinson, 2008b). One well-documented case is the decline of Hawaiian endemic bird species primarily due to their high susceptibility and immunological naivity to *Plasmodium relictum* accidentally introduced with its vector, *Culex quinquefasciatus* (van Riper *et al.*, 1986; Warner, 1968). Numerous factors have contributed to the extinction of 23 out of 70 species and subspecies endemic to the Hawaiian islands, but *Plasmodium relictum* is thought to be the most important one, at least in explaining the extinctions in the 1900s (van Riper *et al.*, 1986). *Plasmodium relictum* continues to limit the recovery of most species (Benning *et al.*, 2002; Atkinson *et al.*, 1995) due to the high susceptibility of native honeycreepers (Atkinson *et al.*, 2000; Atkinson *et al.*, 1995), high transmission of malaria in the lowland areas of the island (Woodworth *et al.*, 2005), and presence of disease-free areas in the highlands that provide a source of immunologically naïve birds to initiate epizootics at lower elevations (van Riper *et al.*, 1986).

Chronic infection with *Plasmodium* spp. may not impact directly on an individual's survival, but it may affect its reproductive success and consequentially limit the population non-directly. However, in the case of the Hawaii amakihi (*Hemignathus virens*) chronically infected parents had the same clutch size, number of nestlings and fledglings than non-infected parents, and their fledglings had the same body mass as those produced by non-infected parents (Kilpatrick *et al.*, 2006).

The energy trade-off between reproduction and the immune system has often been hypothesised but direct experiments are needed to test the hypothesis (Sheldon and Verhurst, 1996). It is essential to establish an increased energetic effort in experimental studies to establish that the trade-off actually occurs (Sheldon and Verhurst, 1996). One such study was conducted on great tit (*Parus major*) clutch size, in which females that had their first two eggs removed, and that consequentially had to lay more eggs, had higher prevalence of *Plasmodium* spp than females in the control group (Oppliger *et al.*, 1996). Males of either group did not show any significant difference in prevalence since their reproductive effort was not increased (Oppliger *et al.*, 1996). Another experiment on great tits also investigated the cost of raising an enlarged brood by removing two chicks, just hatched, from one brood and adding them to another brood, and then measuring the rate of provisioning of the male and female of each dyad (Richner *et al.*, 1995). Males from enlarged broods had higher *Plasmodium* spp. prevalence than males from reduced or unmanipulated broods, while there was no difference among females (Richner *et al.*, 1995). Norris and colleagues (Norris *et al.*, 1994) also showed, through manipulated brood size, that males, but not females, provisioning the enlarged broods had higher prevalence of *Leucocytozoon* spp. than other males. Sheldon and Verhurst (1996) suggested that these experiments were suggestive of a trade-off between energy allocated to raising chicks and ability to fight haemoparasite infections. However, there is also the possibility that males with enlarged brood had to spend more time foraging, and hence they were more exposed to biting mosquitoes (Sheldon and Verhurst, 1996), therefore a measure of the intensity of infection would have given a better idea of the ability of the host to control infection.

The prevalence of *Plasmodium* infection in free-living birds ranges from study to study. In Spain, cirl buntings (*Emberiza cirlus*) presented 36% prevalence of *P. relictum* (based on 111 birds) (Figuerola *et al.*, 1999), a similar prevalence rate was found in collared flycatchers (*Ficedula albicollis*) in Hungary (based on 43 birds) (Szöllősi *et al.*, 2009), but in France a study on house sparrows found prevalence ranging from 40.6 to 58.3 % (Bonneaud *et al.*, 2006) (based on 208 birds) but only 24% in house sparrows in Panama (based on 60 birds) (Martin II *et al.*, 2007) and 11.4% on Mauna Loa, Hawaii (based on

70 birds) (van Riper III *et al.*, 1986). Valkiūnas and Iezhova (2001) found *Plasmodium* prevalence in yellow wagtails (*Motacilla flava*) to be 25% (of 180 birds). The time of the year is important when estimating prevalence, with peaks usually occurring in spring and autumn (Cosgrove *et al.*, 2008) due to the spring relapse and the autumn recruitment of immunologically-naïve juveniles into the population (White *et al.*, 1996). The overall prevalence of *Plasmodium* infection in blue tits (*Cyanistes caeruleus*) in Wytham Wood, Oxford, was 24.4% (based on 886 birds), including peaks of almost 30% in spring and 50% in autumn (Cosgrove *et al.*, 2008). The prevalence in breeding blue tits ranged geographically within a woodland area in Oxford from 10 to 60% (based on 997 birds) (Wood *et al.*, 2007) with an average of 24.4%.

Prevalence may increase with the age of the bird, as new infections accumulate in a population (Wilson *et al*, 2002), although Figuerola and colleagues (Figuerola *et al.*, 1999) did not find any difference in prevalence of *Plasmodium* between young and adult cirl buntings. Ferrell and colleagues (Ferrell *et al.*, 2007) suggested that haemoprotozoan infections can be very acute in young and immunologically naïve juveniles but they did not find a difference between ages in the cases of *Plasmodium* fatalities among 6 zoo birds they analysed. Juvenile birds of native and introduced species on the Hawaian island of Mauna Loa Volcano did not have higher prevalence of *Plasmodium* infection, but up to six times higher prevalence than adult birds, especially in native species, indicating that resistance to the parasite is lower once juveniles are infected (van Riper *et al.*, 1986). Some authors (Ots *et al.* 1998; Norris *et al.*, 2004) have claimed difference in prevalence and intensity between sexes, although this finding has not always been supported (Wood *et al.*, 2008, Martin II *et al.*, 2007; Valkiūnas and Iezhova, 2001).

Leucocytozoon

The genus *Leucocytozoon* comprises vector-borne parasites closely related to the genera *Plasmodium* and *Haemoproteus* (Valkiūnas, 2005). The life cycle has been described by Valkiūnas (2005). Sporozoites enter the avian host from the salivary gland of the vector, usually a biting fly of the order Diptera, when it takes a blood meal, and penetrate hepatic cells to develop into meronts. After increasing in size and reaching maturation,

merozoites and syncytia are formed and released into the blood stream of the host, where merozoites invade erythrocyte and develop into round gametocytes, and syncytia are engulphed by macrophages and form megalomeronts. Megalomeronts contain thousands of merozoites that rupture from the megalomeront and invade lymphocytes and other leucocytes to develop into fusiform gametocytes. The round and fusiform gametocytes (male and female gametes) are infective to the vector, in which they undergo sexual reproduction to form a zygote. The zygote undergoes sporogony in the midgut of the Diptera vector to create sporozoites, which migrate to the salivary duct, ready to be injected into the next host.

Clinical signs of leucocytozoonosis include anemia (Maley and Desser, 1977), anorexia, difficult breathing, and diarrhea (Wobeser, 1997). Infection by *Leucocytozoon* spp. can be diagnosed through screening of a blood smear, where the gametocytes lack the refractile pigments typical of *Plasmodium* and *Haemoproteus* spp., and the fusiform gametocyte distorts the host cell to the point that it is not possible to discern its former identity (Campbell and Ellis, 2007). The pathogenicity of this parasite is considered generally low, although a high mortality rate has been recorded in waterfowl (Wobeser, 1997). Disease is thought to affect mainly juvenile birds (Stuht *et al.*, 1999) and infection is usually subclinical in adults (Forrester and Greiner, 2008; Campbell and Ellis, 2007; Wobeser, 1997).

The prevalence of *Leucocytozoon* spp. in wild birds ranged from 35.4% in blue tits (Merino *et al.*, 2000), 72% *L. dubreuili* and 42% *L. fringillinarum* in birds of the family Paridae and Fringillidae (Deviche *et al.*, 2001), 0.9% *L. majoris* in great tits in Sweden (Allander and Bennett, 1994), 100% *L. ziemanni* in Twny Owls (Appleby *et al.*, 1999), and 3.9% *L. dubreuili* in great tits (Hauptmanova *et al.*, 2002).

The impact of Leucocytozoon spp. on wild bird populations is not clear (Forrester and Greiner, 2008), but mortality events in waterfowl have been reported (Wobeser, 1997), and mortality of pink pigeon squabs in Mauritius have been attributed to *Leucocytozoon marchouxi* (Pierce *et al.*, 1997). Bunbury and colleagues (Bunbury *et al.*, 2007) found

that young pink pigeons in Mauritius had higher prevalence of *Leucocytozoon marchouxi* than adult birds, and that the infection was correlated to reduced survival.

Leucocytozoon spp. have been used as model parasite to investigate the cost of parasitism. In their seminal paper on the cost of parasitism and sexual selection, Hamilton and Zuk (1982) used several parasites, including *Leucocytozoon* spp., to test their hypothesis of bright plumage as indicator of fitness as resistance to parasites. Norris and colleagues (Norris *et al.*, 1994) used *Leucocytozoon* spp. prevalence to test the hypothesis of a trade-off in energy allocation between the ability to fight infection, and breeding effort. Merino and colleagues (Merino *et al.*, 2000) suggested a cost of *Leucocytozoon* spp. based on an medication experiment of wild blue tits. However, the authors considered the combined effect of *Leucocytozoon* spp. and *Haemoproteus* spp. infection, therefore differentiating the effect of the two parasites or a synergistic effect on the host is not possible.

1.2.3 Coccidia

Coccidian parasites is the general term to identify protozoan parasites belonging to several genera, including *Isospora, Eimeria, and Atoxoplasma*. The exact classification is cause for debate and it is based on the morphological characteristics of the sporulated oocysts.

Coccidia are transmitted through the faecal-oral route via the ingestion of oocysts. When the protozoa are ingested by a suitable host, the oocysts excyst in the intestinal tract and release sporozoites, which then invade the epithelial cells that line the mucosa. They undergo merogony (asexual reproduction), and produce daughter cells (merozoites). More generations of merogony increase the number of parasites in the host. The last generation of merozoites penetrate the epithelial cells and initiate gametogony, the sexual phase of reproduction. Gametes fuse to form a zygote, around which an oocyst wall is formed, and the unsporulated oocyst is released, killing the host cells in the process. Oocysts are infectious to the next host only after sporogony (sporulation),

which occurs within a few days of the shedding. Coccidia species can be distinguished in their enteric form by the number of sporocysts present after sporulation. *Isospora* oocysts contain two sporocysts, each with four sporozoites; *Eimeria* oocysts contain four sporocysts, each with two sporozoites; *Caryospora* oocysts contain a single sporocyst with eight sporozoites. (Greiner, 2008).

Coccidiosis is the clinical disease resulted by an infection with Coccidia (coccidiasis), and it is an important disease of domestic fowl and it has economical repercussion worldwide. Coccidiosis is rare in free-ranging birds, and it is usually related to crowding condition, stress, and general captive rearing, since the severity of the disease increases with the increasing ingestion of oocysts. Furthermore, it is an age-dependent disease, in which young and naïve birds are more likely to develop the disease. (Greiner, 2008).

Coccidiosis is generally very rare in wild birds, and only sporadic cases of intestinal coccidiasis have been reported (Ruff *et al.*, 1984). Furthemore, passerines tend to be primarily infected by *Isospora* and *Atoxoplasma*, rather than by *Eimeria*, which is more common in Galliforms (Shirley *et al.*, 2005; Allen and Fetterer, 2002), Anseriforms (Windingstat *et al*, 1980), and Gruiforms (Spalding, Carpenter and Novilla, 2008), but in general species of *Eimeria* do not cause disease in free-ranging birds, and only young birds or adults that are stressed and unhealthy are likely to develop clinical coccidiasis (Yabsley, 2008a).

Clinical signs are not evident during coccidiasis, because at low-intensity infection levels only few epithelial cells are destroyed, and they are quickly replaced. (Greiner, 2008). At moderate to high infection, a large number of cells are destroyed, leading to reduce absorption of water and nutrients, and it can lead to haemorrhage (Greiner, 2008). Affected birds showing symptoms of coccidiasis may pass faeces with blood, show emaciation, loss of coordination, ruffled feathers, and even decreased egg production (Hunt and O'Grady, 1976). Coccidiasis can be sub-clinical, therefore the finding of oocysts in faecal samples does not indicate that the species of Coccidia has caused the death of the individual (Greiner, 2008).

Isospora and *Atoxoplasma*

Isospora species can be distinguished by other coccidian parasites by their oocyst with two sporocysts containing four sporozoites each (Greiner, 2008). They are monoxenous, requiring only one host to complete the life cycle, and most of them undergo their life cycle in the intestinal epithelium of the avian host. *Atoxoplasma* can be identified by the presence of sporozoites in monocytes and lymphocyte in blood smears (Campell and Ellis, 2007).

Recent taxonomic developments placed *Atoxoplasma* and *Isospora* as synonyms (Barta *et al.*, 2005), although the term "*Atoxoplasma*" is still used to identify the extraintestinal asexual phase of some *Isospora* spp that are found in monocytes and lymphocytes in the blood (Campbell and Ellis, 2007). *Atoxoplasma* was formerly identified as belonging to the genus *Lankesterella*, which, however, is heteroxenous (it has two types of hosts in its life cycle) and found in erythrocytes (Levine, 1982).

Oocysts of Isospora need to be aerated for about a week to allow sporulation and hence become infective (Greiner, 2008). Species of *Isospora* that cause atoxoplasmosis undergo merogony in blood mononuclear phagocytes in the mucosa of the gut (Greiner, 2008). Some of these monocytes can be found in the circulating blood, but the final merogony and gametogony stages occur in the intestinal epithelium like in enteric coccidian (Box, 1977). It is impossible to distinguish oocysts of enteric *Isospora* species that cause atoxoplasmosis from those species that remain confined to the intestinal tract throughout their reproductive cycle, and there is often a very weak correlation between faecal oocysts and mononuclear merozoites (Greiner, 2008), although Ball (1998) was able to correlate monocytes infections with oocysts in the faeces of 14.8% of greenfinches (*Carduelis chloris*) in Great Britain. In some birds the number of oocysts shed by adults increase during the nesting season (Greiner, 2008).

The prevalence of atoxoplasmosis in free-living wild birds is very variable. In Hawaii, *Atoxoplasma* prevalence ranged from 0.1% in Japanese white-eyes (*Zosterops japonicus*) to 2.9% in house finches *(Carpodacus mexicanus*), to 8.6% in house

sparrows, up to 17.4% in nutmeg mannikins (*Lonchura punctulata*) (van Riper *et al.*, 1987). The prevalence in house sparrows and tree sparrows (*Passer* montanus) increased to 100% in a Polish study (Kruszewicz, 1991). Microscopy has been, until recently, the only technique available to detect the presence of these protozoa. In recent years Polymerase Chain Reaction (PCR) techniques have been developed to test for the presence of these parasites in the blood, faeces, and tissues of captive birds (Adkesson *et al*, 2005). Atoxoplasmosis is usually a disease of young birds, especially fledglings, and adults are usually not affected (Greiner, 2008).

In house sparrow nestlings analysed in a Polish study the prevalence of *Isospora lacazei* was 11% (of 74 nestlings), and in adults birds it ranged from 55% (of 111 birds), when faeces were collected from nest boxes were examined, to 100% (of 70 birds) when faeces were analysed coming from birds captured in the wild and kept in captivity (Kruszewicz, 1995). In tree sparrows (*Passer montanus*) the prevalence in nestlings was similar to that of house sparrow chicks (10.5% of 57 *pulli*) and of adults kept in captivity (100% prevalence for 40 *pulli*), but lower in faecal samples from nest boxes (32% for 87 birds) (Kruszewicz, 1995). However, it was unclear from the study whether the faecal samples from nest boxes belonged to the nestlings or the adults, and how they estimated how many birds they belonged to, ruling out pseudoreplication. Coccidian parasites were found in 13% (of 237) unhatched eggs of house sparrows, and 7% (of 207) unhatched eggs of tree sparrows (Kozłowski *et al*, 1991). When the age of nestlings and presence of coccidia was analysed in house sparrows, the same authors found the protozoa in 7.7% (of 13) apparently healthy chicks, in 30% (of 10) ill nestlings, and in 50% (of 20) dead pulli of house sparrows up to 5 days old, in 5.5% (of 36) healthy-looking, 21% (of 19) ill and 14% (of 21) dead chicks aged 6-10 days old, and in 6% (of 80) healthy-looking, 15% (of 27) ill and no coccidia in dead chicks older than 10 days. The suggestion was that younger nestlings that are found dead are more likely to have coccidia parasites in their digestive system (Kozłowski *et al*, 1991). In tree sparrows no healthy-looking chicks were positive for coccidia at any age, but 14% (of 7) nestlings aged 1-5 days and 6% (of 53) of pulli older than 10 days were positive for the protozoa (none were positive at the intermediate age), but when dead chicks were examined 53%

(of 17) of 1-5 day old birds, 33% (of 6) of 6-10 day old, but none of the older chicks were positive for coccidia (Kozłowski *et al*, 1991). The authors suggested than the critical period for mortality associated with coccidia parasites is up to 5 days old, and older chicks develop immunity and become carrier (Kozłowski *et al*, 1991).

The shedding of oocyst varies during the day in different avian species, and peaks in the later part of the day have been recorded in the blackbird (*Turdus merula*) (Filipak *et al*, 2009), the serin (*Serinus serinus*) (Lopez *et al*., 2007), the garden warbler (López *et al*., 2007), the blackcap (*Sylvia atricapilla*) (Dolnik, 2006), the dark-eyed junco (*Junco hyemalis*), the house sparrow (Boughton, 1933), the house finch (Brawner and Hill, 1999), the red-legged partridge (*Alectoris rufa*) (Villanúa *et al*., 2006), house and tree sparrows (*Passer montanus*) (Kruszewicz, 1995). This pattern is concurrent in many species, indicating a possible adaptive reason behind it from the point of view of the coccidia spp. One hypothesis advanced by Martinaud and colleagues (Martinaud *et al*., 2009) indicates that this trait evolved to adapt against desiccation and damage done by ultraviolet radiation.

Synchronicity of shedding at the best time for the parasite, known as chronobiological adaptation, is also known in trematodes which shed cercariae at the same time as the definitive host is present in the environment (Théron, 1984), and in some haemoparasites (e.g. *Wuchereria bancrofti* microfilariae) which appear in the peripheral blood of the host at times coinciding with the peak feeding period of its vector (Hawking, 1967).

Parasites have an impact on the individual and the population if they diminish its ability to reproduce and survive (Anderson and May, 1979). *Isospora* spp. have the potential to cause the host's death if oocysts destroy many epithelial cells of the intestine during asexual reproduction, when multiple merozoites are produced which will infect new cells (Box, 1977), as in the case of young cranes (Novilla and Carpenter, 2004). The host specificity of *Isospora* spp. that cause atoxoplasmosis is unknown, and in an experiment

Box (1970) showed the possibility of cross transmission of *Isospora* from house sparrow to house sparrow, but not from house sparrow to canary (*Serinus canarinus*).

The likelihood of transmission of coccidia parasites between birds in the wild is potentially high in flocking species like the house sparrow, which are characterised by gregarious feeding, nesting and roosting behaviours, although experiments to quantify this likelihood are needed. A wide range of bird species are infected with *Isospora* spp., and while most infections do not cause disease and mortality, these can occur in some species.

Coccidiosis can develop if the bird has no previous exposure to the parasite, and the dose of oocysts is high enough (Greiner, 2008; Novilla and Carpenter, 2004). Coccidiosis caused by an *Isospora* sp. was the cause of death of 98 black siskins (*Carduelis atrata*) arrived in Italy from South America (Rossi *et al.*, 1997). The birds arrived showing signs of the infection, and presented severe pectoral muscle atrophy, swollen and congested cloacas with wet faeces on the tail feather (Rossi *et al.*, 1997).

Atoxoplasmosis has been identified as a major problem in the reintroduction captive programmes of the Bali mynah (Partington *et al.*, 1989) caused by the host-specific *Isospora rothschildi* (Upton et al., 2001). It is generally thought that naturally-occurring coccidiasis is asymptomatic in free-living wild birds, but in some cases stress may exhacerbate the infection and pathology (Rossi *et al.*, 1997). Coccidiosis can be associated with anorexia, dehydration and blood loss, and it can increase the susceptibility to other disease agents (Novilla and Carpenter, 2004).

Low-level exposure to oocysts rarely results in death, but it often leads to a chronic infection in adults (Novilla and Carpenter, 2004), which may not confer immunity to subsequent exposure to the same parasites (Hõrak *et al.*, 2004). In young birds the infection can be more severe and the parasite intensity, but not prevalence, may be higher than in adult birds (Lopez *et al.*, 2007; Novilla and Carpenter, 2004). Atoxoplasmosis is sometimes referred to as 'going-light' syndrome (Cooper *et al.*,

1989), because birds may stop eating and lose weight, look lethargic and have ruffled feather, as well as diarrhea (Norton *et al.*, 1993). The clinical signs associated with enteric *Isospora* infection are usually not evident, but they may mimic those of atoxoplasmosis when the disease is present (Greiner, 2008).

The effect that *Isospora* spp. seems to have on the body weight of the affected birds is unclear. blackbirds parasitised with *Ispospora* spp. were not lighter than unparasitised birds (Baeta *et al.*, 2008), but nestlings of house and tree sparrows were 8.8% and 5.4% lighter, respectively, than their non-infected siblings at day 12 in the nest (Kruszewicz, 1995), which is a factor that has been linked to subsequent survival (Seel, 1970). Infection with *Isospora* spp. is associated with loss of body mass through reduced nutrient uptake (Hõrak *et al.*, 2004).

Nutrients such as carotenoids cannot be synthesized by vertebrates, which must acquire them from their food (Olson and Owens, 1994). In species which rely on carotenoids for their plumage colouration (yellow, orange, and red), such as finches, this may lead to changes in appearance of the individual bird and may decrease their mate attractiveness and reproductive success, in a classic example of parasite-mediated sexual selection (Hamilton and Zuk, 1982). A study on greenfinches (*Carduelis chloris*) showed that in males infected with *Isospora* spp. oocysts their tail feathers contained 52% less carotenoids than male birds that were kept parasite-free (Hõrak *et al.*, 2004). In American goldfinches (*Carduelis tristis*) parasitized males also showed significantly less bright carotenoid-based plumage and bill colouration than unparasitised males, but melanin-based colouration remained unaffected by the parasites (McGraw and Hill, 2000). Melanin is synthesized from essential amino acids that are basic components of foods (Fox, 1976), and deposition of pigments in feathers seems to be genetically controlled in some circumstances (Norris, 1993; Møller, 1989). In male house sparrows the bib badge size during moult was correlated with levels of circulating blood protein (Veiga and Puerta, 1996), indicating a role as signal of quality of the individual (McGraw and Hill, 2000).

Coccidia can also affect the structural plumage colouration (ultraviolet, blue, and green) of males, used by females as indicator of male quality (Doucet, 2002). In the blue-black grassquit (*Volatinia jacarina*) Costa and Macedo (2005) found a negative correlation between the coccidian oocyst count and blue-black plumage coverage, as well as decreased body weight in infected individuals compared to non-infected ones. In an experiment aimed at assessing the role of *Isospora* spp. on carotenoid levels and related traits in blackbirds (*Turdus merula*), two groups of birds were inoculated with *Isospora* spp. oocysts, and two groups were kept as control (Baeta *et al.*, 2008). One of the inoculated groups and one of the control groups were also given extra carotenoids in the diet (Baeta *et al.*, 2008). Birds that were infected but had received extra carotenoids had higher plasma carotenoid concentration and unchanged colour intensity of their orange bill compared to the beginning of the experiment, while birds infected but that had not received extra carotenoids had paler bills but no changes in circulating plasma carotenoid levels. Males that were not infected and received extra carotenoids had significantly redder bills (Baeta *et al.*, 2008). The peak shedding of oocyst occurred two weeks later in the carotenoid-fed males than in the non-fed males, although no treatment affected the body mass of the birds (Baeta *et al.*, 2008).

The immuno-enhancing properties of carotenoids have been addressed in some studies (McGraw and Ardia, 2003) with mixed results. In an experiment on blackbirds, carotenoids failed to prevent coccidian parasite multiplication in birds provided with extra carotenoids, but these nutrients were effective in slowing down the replication of the parasites, and males provided with carotenoids had a peak of infection about two weeks later than males without the supplement (Baeta et al., 2008). Coccidian parasites are known to produce free radicals (Allen, 1997), and affect the immune system of the host (Hõrak *et al*, 2004; Smith *et al*, 2002), but Baeta and colleagues (2008) did not find a difference in circulating levels of carotenoids between parasitised and unparasitised male blackbirds.

Infection with coccidia parasites can be associated with other pathogens, such as *Candida* spp. and bacteria from the *Enterobacteriaceae* family, which Kruszewicz

(1995) found, were isolated in *Isospora* spp infected house sparrow nestlings twice as often than in non-infected *pulli*, while *Staphylococcus* spp. were present in *Isospora*-infected tree sparrow nestlings twice as often as in non-infected chicks.

Eimeria

Avian *Eimeria* spp. generally infect and develop in the intestinal epithelial cells of the host, where the asexual and sexual phases of replication, together with the formation of the oocyst, take place in the nucleus or cytoplas of the host cell (Yabsley, 2008). The extraintestinal infection of *Eimeria* has only been recorded in renal epithelial cells, with few noticeable exceptions, such as the multiple organ infection in the cranes (*Grus* spp) by *Eimeria gruis* and *Eimeria reichenowi* (Spalding *et al.*, 2008).

Eimeria spp. have a direct life cycle, and hosts become infected through the faecal-oral route by ingestion of sporulated oocysts. Oocysts are very resistant, and they can tolerate dessication and freezing, and hence persist in the environment . When the oocyst is ingested by an appropriate host, it ruptures releasing sporocysts which in turn rupture and release sporozoites. In the intestinal epithelium, sporozoites transform into trophozoites, which replicate asexually to create meronts. These in turn will undergo merogony to create merozoites, which break out of the host cell and invade other cells, to undergo more cycles of merogony and eventually gametogony. During gametogony, merozoites become either macrogametocytes (female cell) or microgametocyte (male cell). Microgametes exit the cell and enter cells where macrogametes are present, and fertilization occurs. A fertilized macrogamete develops walls and becomes an oocyst, which is then released in the faeces of the host (Yabsley, 2008).

1.2.4. Helminths

The term "helminths" refers to a group of invertebrates which share characteristics such as an elongated body, and creeping movements- essentially the 'worm' and flukes - but differ greatly in the taxonomy. They are parasites and may affect the health and fitness of the carrier (Zucca, 2000).

Most helminthes are intestinal parasites, but birds can also host helminthes in the air sacs, kidneys, eyes, and vascular system (Janovy, 1997). They are transmitted through ingestion of eggs or larvae, or indirectly through ingestion of infected intermediate hosts (Janovy, 1997). Avian helminths include cestodes (tapeworms), nematodes (roundworms), trematodes (flukes) and acanthocephalans (throny-headed worms) (Janovy, 1997).

Cestodes (tapeworms) are the most common helminth parasite found in birds (Rausch, 1983). They are most commonly found in the intestine and they can be recognized by their segmented appearance (McLaughlin, 2008). Apparently healthy birds can harbour hundreds of cestodes (Bush, 1990), and infected birds rarely show clinical signs. Cestodes are not considered to be pathogenic or a threat to avian populations under normal circumstances (Haukos and Neaville, 2003; Delahay, 1999; Greve, 1986) unless they are present in really elevated numbers (Greve, 1986) or in food-deprived and malnourished individuals (Wobeser, 1981).

Nematodes of the genera *Dispharynx, Echinuria,* and *Streptocara* parasitise the proventriuculus and the gizzard of many avian taxa and the presence of eggs in faeces may be used to detect the infection in live birds (Carreno, 2008). The presence of *Dispharynx* spp. has been reported in domestic fowl in the Galapagos Islands, raising concern over a potential transmission to endemic birds (Gottdenker *et al.*, 2005). Tracheal worms are strongylid nematodes that infect the airways of birds, in particular fowl and passerines (Fernando and Barta, 2008).

Clinical signs are apparent at low intensity of infection, and they have been found in wild birds showing signs of respiratory distress (Fernando and Barta, 2008). Pheasants seem to be particularly susceptible to infection, and young birds in particular can show signs such as gaping, coughing, and they may stop drinking; in severely affected individuals the infection may deteriorate and cause death (Clapham, 1934), but adult birds show few clinical signs (Fernando and Barta, 2008). *Ascaridia* nematodes occur primarily in the gastrointestinal tracts of their host (Fedynich, 2008). Their pathogenicity

varies according to species and interaction with the host, but their impact on host population has not been clearly assessed because dying or sick birds are more likely to be predated upon (Hudson *et al.*, 1992a) and hence are rarely found for examination (Fedynich, 2008). However, Hudson and colleagues (1992b) found that the nematode *Trichostrongylus tenuis* had a regulatory effect on the red grouse population via decreased breeding success and winter survival. Infection with capillarid nematodes is very common, but its impact on wild bird populations is thought to be non-negative (Yabsley, 2008).

Trematodes, or flukes, are flat, leaf-like worms that develop in most cases in the intestine of the host but can also migrate to other body systems (Huffman, 2008). Their pathogenecity is generally low, but some species can have debilitating effects and consequentially predispose the host to other pathogens (Huffman, 2008).

Acanthocephala helmiths are characterised by the thorny appearance of their holdfast organ (Richardson and Nickol, 2008). The pathogenicity of this phylum is still under debate, as high parasitic intensity may not cause any ill effects (Moore and Bell, 1983), but low intensity in some cases has been reported to have severe adverse effects (Richardson and Nickol, 2008).

1.3 AIMS

The aim of the work described in this thesis was to investigate whether or not disease, particularly infectious disease, might play a role in the observed declines in sparrow populations in London. The approach used was to make use of an ongoing RSPB study of sparrow populations at various sites in London, some undergoing population declines and some not, and some with supplementary feeding and some not. In particular, the aims were:

1. To investigate the prevalence, and where possible the intensity, of targeted infectious agents and parasites at the various sites.
2. To investigate markers – in particular haematological markers – of infectious diseases.
3. To investigate variation of infectious agents and parasites across years and seasons, and between age, sex, condition, breeding status, and moult status of infected birds.
4. To compare differences in prevalence and intensity of targeted infectious agents and parasites between: sites that were declining or non-declining, and sites that were provided with supplementary mealworms or not supplemented.
5. To investigate survival of house sparrows in relation to the prevalence and intensity of infectious agents and parasites.

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Chapter 2

GENERAL METHODOLOGY

2. GENERAL METHODOLOGY

2.1 STUDY AREA

2.1.1 The RSPB London House Sparrow Project

The Royal Society for the Protection of Birds (RSPB) carried out the London House Sparrow Project from 2004 to 2009, encompassing 66 sites across Greater London (Fig. 2.1) chosen for their remaining colonies of house sparrows. The aim was to test the role of lack of invertebrates in the decline of sparrows in the capital, following a study in Leicester (Peach *et al.*, 2008; Vincent, 2005) in which it was found that chicks were more likely to starve if their diet contained "low-quality" items, such as bread and vegetable matter instead of protein-rich insects such as aphids. The RSPB set up an experiment in which 3 garden owners at each of 33 sites were asked to supply birds with 100g of meal worms, provided by the RSPB in single-dose tubs, twice a day during the breeding season (April to August) using special feeders (Fig. 2.2). Those sites are hereafter referred to as 'Fed Sites'. The other 33 sites were not provided with mealworms, and they are hereafter referred to as 'Unfed Sites'. Regardless of the feeding experiment the households generally provided seeds and peanuts as part of a general personal bird-feeding practice. This was unavoidable since volunteers that agreed to take part in the experiment had a prior interest in garden birds and associated feeding. The bias between sites was thought to be negligible because all households provided bird food in addition to the RSPB mealworms.

The RSPB conducted a survey to establish the size of the breeding population of house sparrows. The survey was conducted twice a year, in March/April and in April/May, in the early morning, using fix transects to count male house sparrows, distinguishing between chirping and non-chirping males which at those times of the year are very vocal, while females are inconspicuous and may be underrepresented in the survey. A second survey was carried out in August to check for fledged juveniles and thus for productivity of each site.

2.1.2 Study sites

All sites considered in this study were in the Greater London Area, defined as the area included within the M25 motorway. A subsample of the 66 RSPB London House Sparrow Project sites was selected for this disease study, taking into account time, person-power, and limitations for catching birds intrinsic to the site characteristics. All sampling was carried out in private gardens.

Table 2.1. Study sites – coordinates, population trend and site code.

Latitude	Longitude	Population trend	Site code
51:28:07N	0:11:17W	Declining	31S
51:22:00N	0:12:36W	Declining	73S
51:31:37N	0:28:47W	Declining	51N
51:35:08N	0:13:21E	Non-declining	86N
51:33:45N	0:23:07W	Declining	85N
51:34:33N	0:01:15W	Non-declining	67N
51:21:54N	0:18:35W	Non-declining	40S
51:36:38N	0:04:40W	Non-declining	33N
51:27:05N	0:02:08W	Declining	59S
51:23:20N	0:14:57W	Non-declining	37S
51:27:53N	0:13:25W	Declining	79S
51:25:22N	0:30:02W	Declining	98N

Twelve sites (termed "intensive") (Table 2.1) were selected and paired up so that each pair would experience the same feeding treatment: i.e. either both received mealworm ("Fed") or both did not ("Unfed"), but opposite house sparrow population trends (one site was stable or increasing, termed "Non-declining", the other 'Declining'), based on data provided by the RSPB since the beginning of their project in 2004. The sites in the dyad were selected so that they had a similar population size at the beginning of this study, and similar habitat including vegetation and housing type.

A further set of 23 'non-intensive' sites were chosen to be added to the 12 intensive sites above for taking swabs of bird tables (see section 2.2.2 for more details) to monitor the presence of pathogenic bacteria at feeding stations. These 23 sites were chosen randomly in regard to location, population trend, and feeding regime, but the ultimate choice depended on the availability of the garden owners to take swabs.

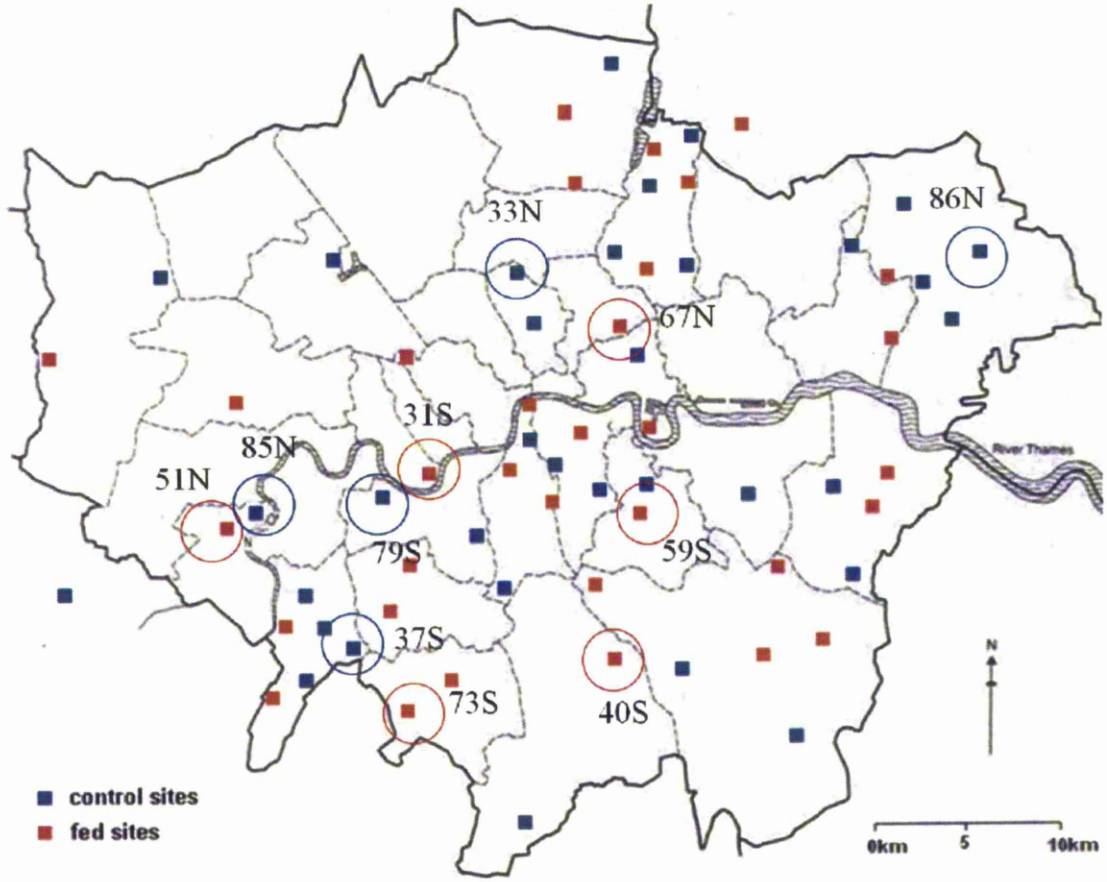

Fig. 2.1. Map of the 66 sites considered in the RSPB study. Intensive study sites are circled, red circles indicate fed sites, blue circles unfed sites. Modified from an original created by W.Peach.

2.2 DATA COLLECTION

2.2.1 Bird ringing

Bird ringing is undertaken to mark birds as individuals by fitting a lightweight metal ring with a unique alphanumerical code on one of the bird's legs. The scheme as we know it started in 1909 and it is now coordinated in Great Britain and Northern Ireland by the British Trust for Ornithology (BTO) on behalf of JNCC. The BTO issues bird ringing permits and rings, and a license is granted after a training period with a licensed trainer. There are several methods for trapping birds, all requiring a license under the Wildlife and Countryside Act 1981. The most widespread way to trap passerine birds is with a mist net (Fig.2.2) erected between poles. This procedure is very safe for the birds, and it allows flexibility in the location of the nets, but it is non-selective in the species of birds caught, therefore in this study other species were caught in addition to house sparrows, which was, however, the only species sampled.

All birds were extracted by a licensed ringer, and transported in a cloth bird bag for few metres from the net to the processing area. Processing involved species identification, fitting of an appropriate-size ring (or reading the code of the existing ring if a bird was a re-trap), identifying age and sex when possible, and taking basic biometric measurements such as wing length, tarsus length, body mass, and moult score. Each house sparrow was also checked for presence of ticks, lice, and any external lesions such as pox lesions, and a sample of faeces and blood was also collected (see section below for more details). Blood samples were taken under licence from the Home Office. House sparrows were also fitted with 3 plastic rings of different colours so that each individual could be identified from its unique colour combination without being re-caught. The RSPB conducted resightings surveys of the colour-ringed house sparrows to ascertain survival. Each bird was released after processing, within maximum 30 minutes of capture, which is in line with the BTO rules (Redfern and Clark, 2001).

Each site was visited once every 6 weeks (twice per season), throughout the year, with the aim of sampling at least 10 new house sparrows per visit. In circumstances when this minimum number was not obtained in a single visit, a second visit was attempted between 1 to 5 weeks later, according to the availability of the garden owner, the weather, and the schedule of sampling of the other sites. Birds were sampled for blood at intervals not shorter than 28 days, following Home Office rules.

Recapture rate was low, as expected from a species which becomes 'net-shy' very easily: of the 262 adults birds caught, 31 were caught more than once, while of the 227 juveniles caught, six were caught again as juveniles, and 17 were caught again as adults. Some birds were re-captured more than twice: seven adults and four juveniles were caught three or more times, although in the case of juveniles, they were all re-caught the third time as adults.

Fig. 2.2. Mist-net and RSPB mealworm feeder in one of the study sites. (Photo: Daria Dadam).

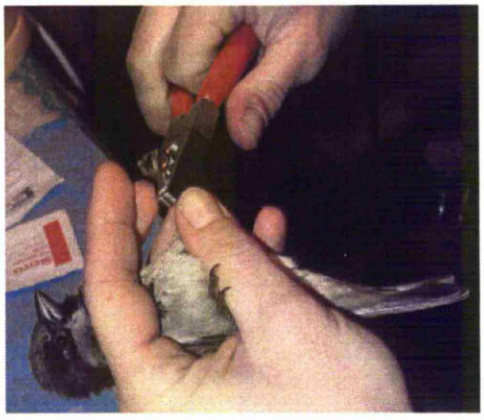

Fig.2.3. Fitting a ring on a male house sparrow (Photo: Mark Jones).

Ageing and sexing house sparrows

The age and sex of birds can be done in many cases by observing the plumage or, during the breeding season, the presence of external reproductive organs such as the cloacal protuberance, as outlined for passerines by Svensson (1992). The ageing of passerines is based on their moulting strategy, which can vary. The three main ones are describes by Ginn and Melville (1983):

1) Moulting after the breeding season before winter (post-nuptial/post-juvenile moult)
2) Moulting before the breeding season in spring (pre-nuptial moult)
3) Moulting both after the breeding season and before the following one in spring

Furthermore, birds can have a partial moult (hence retaining some feathers from the previous calendar year), a complete moult (with all feather changed and hence no contrast visible between different generations of feathers), or even an arrested moult (stopping the moult at any point and then re-starting it at a later date). Finally, adult and juvenile birds (birds in their first calendar year) of the same species can adopt different strategies. In the house sparrow both adults and juvenile birds undergo a complete post-

nuptial/post-juvenile moult at the end of the summer – beginning of autumn. The result of this strategy is that both adults and juveniles have a complete set of new feathers once the annual moult cycle is complete, and their ages are henceforth no longer discernible. This means that from about October onwards birds born in the current calendar year (juveniles) and adult birds (birds born at least in the previous calendar year) are not distinguishable, unless they were ringed before or during the moulting cycle and re-trapped afterwards.

A further feature that needs to be carefully examined is the quality of the feathers to distinguish juvenile birds prior to their post-juvenile moult from adult females, since juvenile house sparrows of both sexes have the same colouration and plumage pattern as adult female house sparrows. Juvenile birds in their pre-moulting plumage look fresher than adult females, the plumage of which would be older because grown the previous year, and would look abraded by the friction of entering and exiting the nest cavity. However, care must be taken when an older juvenile (born early in the season but still in his fledging plumage) is examined, because its plumage may look slightly worn, since juvenile feathers are of lower quality compared to adult feathers (Svensson, 1992). Pulli in the nest have only 3 weeks to grow all their feathers before fledging, so they sacrifice quality of the feathers which abrade faster than adult feathers would.

2.2.2 Collection of blood samples, faecal samples, and oral swabs

Blood and blood smears

After each house sparrow was processed (see section above) and before it was released, a sample of blood was taken from the brachial vein of only one wing. This process was performed under Home Office Licence. The bird was held firmly with one of the wings gently stretched out, and the vein was located and swiped with a mono-use pre-injection swab (Sterets Pre-injection swab, Medlock Medical), which had the dual function of disinfecting the area and rendering the vein turgid (G. Vaschetti, *pers. comm.*) and hence easier to locate. The vein was then pierced using a 27-gauce mono-use syringe needle (Becton Dickinson Microlance), which was inserted slightly in the vein at an

approximately 45 degree angle, with the slate facing upwards, using great care not to pierce the vein side-to side, nor to pierce the nearby tendons. The needle was inserted and withdrawn after a moment of hesitation, which prevented the vein from collapsing and haematoma formation (Gabriella Vaschetti, *pers. comm.*).

The drop of blood forming outside the puncture spot was collected with a microscope cover-slip (VWR International) and used to make a blood smear on a pre-washed and pre-polished glass slide (Menzel-Glaser), which was labelled with the bird ring number, the date and site code, then air-dried and subsequently transported to the laboratory for fixing and staining (see section 2.3.1). The rest of the blood was collected using a heparine-coated capillary tube (Spirocap 100 mm, Bilbate Ltd) and a EDTA-coated capillary tube (Micro-haematocrit Tubes 100mm, Bilbate Ltd) if enough blood was available. Capillary tubes were sealed with Cristaseal (Hawksley) and labelled with the ring number and date. A cotton ball was applied to the vein until haemostasis was achieved.

Fig. 2.4. Blood sampling: a) The brachial vein is disinfected with a mono-use pre-injection swab, which also makes the vein turgid (b) and easier to see. After puncturing the vein with a sterile single-use needle (not shown), a blood smear is made (c) and some blood is collected in a heparin-coated capillary tube (d). (Photos: a) and b) by Eleanor Page; c) and d) by William Haines).

Welfare of the bird

After the blood was collected, the puncture wound was covered with the sterilising swab and a piece of clean cotton wool; a slight pressure was applied to stop blood flowing out of the puncture, and the wing was closed so that pressure on the cotton wool remained for approximately a minute. The vein was then checked to ascertain that blood had stopped flowing out of the puncture, and the wing was moved slightly up and down to make sure that wing movement would not re-start the blood loss. The quantity of blood that was collected overall never exceeded 200 µl, and although the quantity of blood that

came out of the puncture spot varied greatly from one individual to the other, it generally averaged around 120 µl per individual. These quantities are much lower than the maximum allowed by the Home Office, which is 10% of the total blood volume, which is estimated to be 10% of the body weight of the bird (Campbell, 1995), hence the total volume that could be extracted from a bird should be no more than 1% of its body weight (260 µl for an average 26g house sparrow). Following Home Office rules, birds were never re-sampled at intervals shorter than 28 days. In the rare occasions when, following the puncture, no or very little blood had flowed out of the vein, the bird was released without further attempt to collect blood, because the welfare of the bird was always of paramount importance.

Faecal samples

Faecal samples were collected directly from the bird when the sample was excreted as a faecal sac (often sticking to feathers at the vent), or collected from the bird bag where the bird had been waiting to be processed. Each bird bag was used only once in each bird ringing session, and subsequently washed at 90 degrees to kill any bacteria. The faecal sample was stored in a 1.5ml plastic tube which was labelled in the field with the ring number of the bird, the site, and the date. In some cases the size of the scat was too small for collection, and a sterile swab with charcoal medium (Transwab for aerobes and anaerobes, Medical Wire % Equipment Co. Ltd) was used instead to collect sample from the bag to test for bacteriology only. Faecal samples and swabs were stored in the laboratory in a fridge at 4°C for maximum up to 24 h before bacteriological analyses.

Swabs of Bird Tables

Bird tables of intensive and non-intensive sites were swabbed with a sterile swab (Transwab for aerobes and anaerobs, MW&E). Intensive sites were sampled once a week, while non-intensive sites once every fortnight from March 2007 to August 2008. Garden owners were trained to take swabs using a standardised and repeatable methodology (Fig. 2.5) and asked to mail the swab to me within 24 h of the sampling using the pre-paid, pre-addressed envelope. Detailed instructions on how to perform the swab (inclusive of a diagramme of the movement to perform across the bird table),

together with swabs, plastic bags, addressed padded envelopes, and disposable gloves were provided for further reference. Once received, the swab was either stored in the fridge or cultured straight away, depending on the day of the week. When the garden contained only bird feeders, but no birds tables, the garden owner was asked to take a swab of the area underneath the bird feeder, which was where faecal samples and food spillage would occur, and where ground feeding species such as house sparrows often eat. The area underneath the bird feeder was similar to that of a standard bird table, and the methodology was the same as for bird tables with the feeder at the ideal centre of the swabbing area (Fig. 2.5).

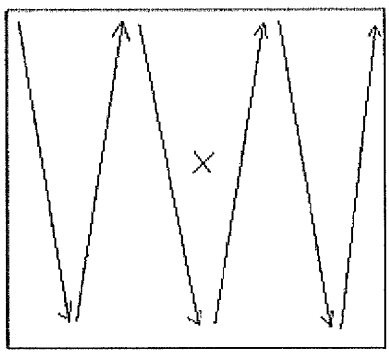

Fig. 2.5. Pattern of feeding station swabbing motion. The "X" in the centre indicates the ideal position of the bird feeder in gardens where a bird table was not present . (Adapted from Pinches, 2002).

Oral swabs

Oral swabs were performed by inserting the tip of a sterile swab (Fine-tip sterile swab, MW&E) in the oral cavity of the bird, using great care not to damage the inside of the epithelium or any organs such as the trachea. The swab was then put in its tube (with no medium) and labelled with the ring number of the bird, the name of the site and the date. The swabs were stored at room temperature until back in the lab, where they were stored in a -24°C freezer for subsequent PCR analysis to identify *Trichomonas* spp, although these tests were not performed as part of this project due to time constraint.

2.3 LABORATORY ANALYSES

2.3.1 Haematology

Fresh blood from the capillary tubes were analysed on the same day for total leukocyte count (or total White Blood Cell count, WBC), total erythrocyte count (or total Red Blood Cell count, RBC), packed cell volume (PCV), fibrinogen, haemoglobin (Hb), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), and the mean cell volume (MCV). Haematological tests were all performed manually apart from haemoglobin test (see relevant section below) because avian erythrocytes and thrombocytes are nucleated and automated counting methods used to count leucocytes would have not distinguished between erythrocytes, thrombocytes and leucocytes (Campbell and Ellis, 2007). Blood smears were fixed and stained to perform differential leucocyte counts and checked for presence of Haemoparasites (see section below).

Total leukocyte and total erythrocyte counts

The total leukocyte count was calculated by diluting 20µl of fresh heparinised blood in 380ul of Rees and Ecker solution (to give a 1:20 dilution) to colour the cells (Dacie and Lewis, 1991). The blood and stain quantity were measured with calibrated epperdorf pipettes. Each solution was put in a 5ml plastic tube which was covered with a piece of parafilm and shaken to mix the blood and the solution evenly. It was then let to set for 10 minutes, shaken again, and the mixture loaded in a Neubauer-ruled hemacytometer (Hawksley). The counting chamber was put on top of moist paper in a box to prevent the solution from drying out (Mike Hart., *pers. comm.*.) while it set for 10 minutes before placing it under the microscope and counting cells at 40x magnitude. The area considered for leukocyte counts is shown in Fig 2.6.

The same solution was then further diluted with 3.6 ml of Rees and Ecker solution to make a 1:200 dilution to count erythrocytes. The same procedure was used for loading the counting chamber as described for leukocytes, and the area of the chamber considered is illustrated in Fig 2.6. Cells found on the upper border of a quadrant were

considered inside the quadrant below, cells found on the left border of a quadrant were considered in the quadrant to their right.

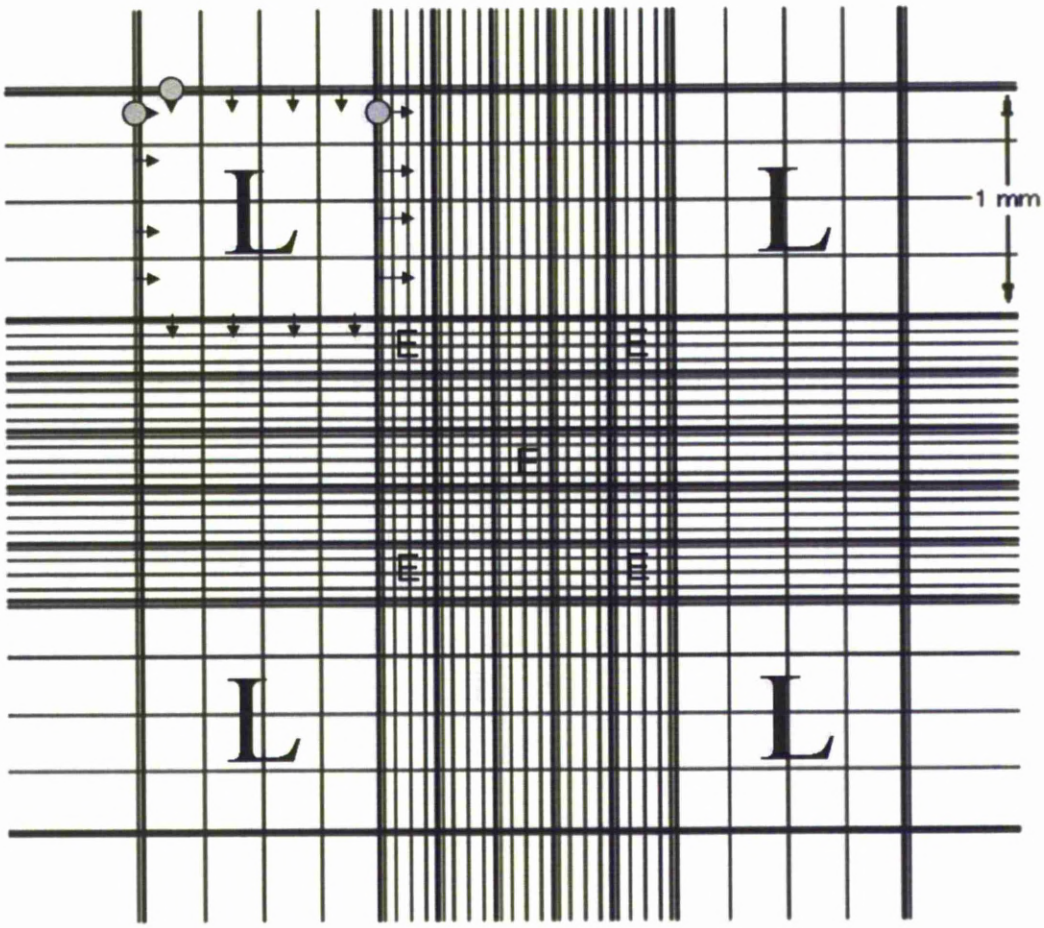

Fig. 2.6. Quadrants of the Neubauer Chamber used to count leucocytes (white blood cell, "L") and erythrocytes (red blood cells, "E"). Cells (closed circles) found on the upper border of a quadrant were considered inside the quadrant below, cells found on the left border of a quadrant were considered in the quadrant to their right. (Modified from Carl Roth, www.carlroth.de) .

The total number of cells in each individual was obtained with the following formulae (Dacie and Lewis, 1991):

$$\text{Total Leukocyte count (l)} = \frac{Cells\ counted}{4x0.1} x\,10^6 \qquad \text{Equation 1}$$

$$\text{Total Erythrocyte count (l)} = \frac{Cells\ counted}{4x0.1} x\,10^9 \qquad \text{Equation 2}$$

Packed Cell Volume / Haematocrit (PCV)

Blood was transferred from the EDTA-coated capillary tubes to a non-anticoagulant haematocrit capillary tube, which was spun in a micro haematocrit centrifuge at maximum speed for 5 minutes. The percentage of erythrocytes to the total blood was calculated using an haematocrit reader. The limited quantity of blood available for the tests meant that PCV could only be measured on blood collected in EDTA-coated capillary tubes, because the process was part of the test for fibrinogen (see below).

MCV, MCH, MCHC

The mean corpuscular volume (MCV) is the measure of the average volume of individual erythrocytes (Samour, 2006) and it is calculated (Campbell, 1995):

$$\text{MCV (fl)} = \frac{PCV(\%)}{RBC} x\,10 \qquad \text{Equation 3}$$

The mean corpuscular haemoglobin (MCH) is the average haemoglobin content per erythrocyte (Samour, 2006) and it is calculated (Campbell, 1995):

$$\text{MCH (pg)} = \frac{Hb}{RBC} x\,10 \qquad \text{Equation 4}$$

The mean corpuscular haemoglobin concentration (MCHC) is the measure of the volume within the erythrocyte occupied by haemoglobin and it is calculated (Campbell, 1995):

$$\text{MCHC (gm/dl)} = \frac{Hb}{PCV(\%)} x\,100 \qquad \text{Equation 5}$$

Fibrinogen

Fibrinogen was tested by hand using the heat precipitation method described by Millar and colleagues (Millar *et* al., 1971). The technique involved using EDTA-coated blood in an haematocrit capillary tube, spinning it at 14,000 RPM for 5 minutes, immersing the column of blood in a water bath at 56°C for 3 minutes, re-spinning it at 10,000 RPM for a further 5 minutes, and then check the amount of fibrinogen under a light microscope fitted with a vernier scale, and a x10 eye piece. The eye piece graticule was used as reference point to take the readings of the various blood columns components (Fig.2.7) using the vernier scale.

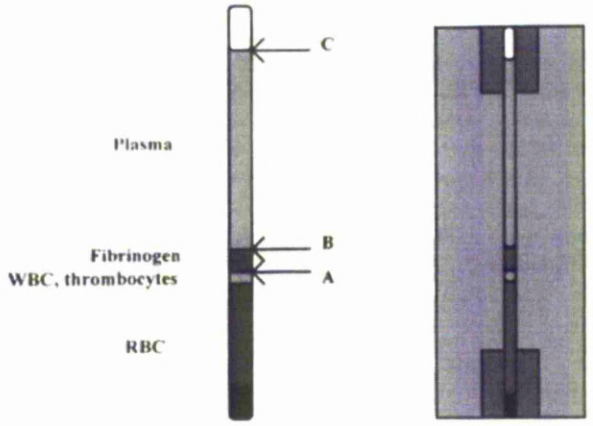

Fig. 2.7. Schematic view of components measured to assess fibrinogen quantity from a blood sample. (Reproduced from Samour, 2000).

The amount of fibrinogen is then calculated as:

$$\text{Fibrinogen} = \frac{B - A}{C - A} x100 \ \text{g/100ml} \qquad \text{Equation 3}$$

Haemoglobin

The quantity of haemoglobin was calculated using the portable haemoglobinometer HemoCue Hb 201+ system (HemoCueAB). The machine deploys special single-use cuvettes which have dried reagents that dissolve in contact with the blood that is then analysed photometrically by the machine, which was calibrated frequently with its reference cuvette. Ten microlitre (10 ul) of blood was measured with a calibrated pipette and inserted in the microcuvette following instructions, and avoiding any air-bubble. The cuvette was inserted in the instrument that expressed the results in grammes of Hb per litre of blood. This method is highly repeatable (Lardi *et al.*, 1998; *personal observation*) and comparable with other methods of estimating Hb (Lardi *et al.*, 1998). If blood had haemolysed or clotted in the capillary tube it was not used for the analysis.

Staining of Blood Smears

Blood smears were fixed in 100% Methanol for 3 minutes as soon as possible on the return from the field (usually within 6 hours of preparation), and then left to air dry. They were subsequently stained either with an automatic machine at Greendale Diagnostic Laboratories (Kent, UK) or by immersing the slides in a Giemsa Stain for 5 minutes, followed by 20 minutes in May-Grunwald stain, and then left to air dry.

Differential Leukocyte Count

Differential white blood cell count was performed on a stained blood smear using a x1000 magnification microscope lens with oil immersion. Each type of white blood cell (basophil, eosinophil, heterophil, lymphocyte and monocytes) was counted separately using a 6-position tally counter (one button per each type of cell) that alerted with a sound when the total of 100 cells has been counted. This method gave the percentage of each type of leukocyte present in the blood, and when multiplied by the total WBC

count, it also gave the amount of each type of white blood cell in the blood, and the Heterophil/Lymphocyte ratio could also be estimated.

Detection and quantification of Haemoparasites

Stained blood smears were screened first at x10 magnification to identify the presence of any microfilaria , then 10,000 erythrocytes were screened for presence of *Plasmodium* spp, *Haemoproteus* spp, and *Leukocytozoon*, spp, by screening 100 fields of view at x1000 magnification with oil immersion. Parasite numbers and parasite intensity were expressed as number of each parasite species per 10,000 erythrocyte. *Atoxoplasma* spp haemoparasites were calculated per 100 leucocytes using a counting chamber at the same time as the leukocyte total differential count was performed.

All parasites were identified using the guidelines suggested by Campbell and Ellis (2007) and Valkiūnas (2005). New techniques using Polymerase Chain Reaction (PCR) have been developed in recent years for detecting *Haemoproteus* and *Plasmodium* in blood samples to supplant or integrate screening of blood smears (Cosgrove *et al.*, 2008; Wood *et al.*, 2007), and some authors have claimed a higher prevalence of haemosporidian parasites when PCR techniques have been used (Ricklefs and Sheldon, 2007), but other authors have disagreed (Jarvi *et al.*, 2002). In this study, data on haemosporidian prevalence and intensity were collected using only blood smears because, even acknowledging the higher detection power of the newest PCR techniques, very low intensities that may be missed by screening only blood smears are unlikely to be pathogenic (for example Valkiūnas and Iezhova, 2001). Low intensities may subsequently developed into more pathogenic stages, but a blood sample can only provide a snapshot of that specific time of sampling, so the course of the infection thereafter can only be speculated, unless the individual is resampled at close intervals.

2.3.2 Bacteriology

Bacteriological tests were performed on fresh faecal samples from house sparrows or from swabs of faeces taken from clean bird bags in instances when not enough sample of faecal matter was available for collection. Swabs from bird-tables were also tested for bacteriology using the same methodology.

The analyses were aimed at identifying primarily the presence of *Salmonella* spp. and *Escherischia coli*, but presence of *Campylobacter* spp was also routinely tested. Faecal samples were initially plated on xylose-lysine deoxycholate (XLD) agar (QCM Laboratories), Colombia blood agar (CBA) supplemented with 5% sheep blood (QCM Laboratories or E and O Laboratories) under aerobic conditions for 48 hours at 37°C , and selenite Salmonella-enrichment broth (QCM Laboratories or E and O Laboratories) incubated under aerobic conditions for 24 hr at 37°C followed by subculture on XLD agar under aerobic conditions if the broth showed suspected *Salmonella* spp. growth (the broth would turn pink in colour and cloudy in texture). Campylobacter-free blood medium (QCM laboratories; modified CCDA-Preston) was incubated under microaerophilic conditions at 37°C . Agar plates and broth vials were labelled with the ring number of the bird, the date of the culture, and a laboratory code number. Using a sterilised loop a sample of faeces was smeared on each plate, rotating the plate and smearing it at 30 degree angle to obtain single colonies. The loop was sterilised and cooled down again before the final application. The same procedure was followed for the second plate, for which a new aliquot of faecal sample was used.

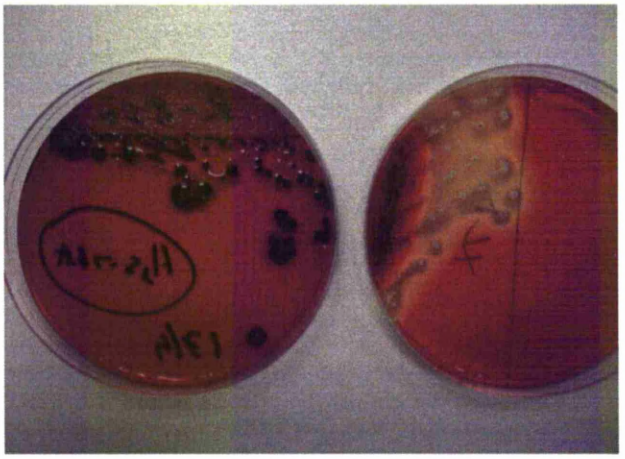

Fig. 2.8. CBA and XLD plates showing a suspected *Salmonella* spp growth, note the black colonies on pink substrate. (Photo: Daria Dadam).

After incubation the plates were checked for any black colonies that may have been indicative of *Salmonella* spp. growth (Fig. 2.8), and in the case of mixed colonies the black ones would be isolated and re-cultured on another XLD agar plate. When a single *Salmonella* spp-looking colony was isolated, it was tested to check the exact identity of it by either Shaheed MacGregor or Shinto John .

2.3.3 Faecal parasitology

The standard flotation technique described in many veterinary textbooks (e.g. Samour, 2000) involves a known weight of faecal matter to relate to the count of oocysts. This technique is, however, often impractical in wild bird studies, especially those involving small passerines where the amount of faecal sample is difficult to weigh (Dolnik, 2006). An alternative method is to use a droplet of faecal matter, which has proved to yield consistent and comparative results to the flotation technique (Dolnik, 2006; Filipiak *et al.*, 2009).

Faecal samples were screened for presence of gastro-intestinal parasites under a light microscope. The faecal pellet was dissolved into seline solution in a volume 1:2 to standardise the dilution. The faecal matter and the seline solution were mixed by shaking

vigorously the tube containing the solution. Two drops of the diluted faecal matter were placed on a microscope slide using a single-use disposable pipettor (VWR), and then covered with a microscope coverslip. The area under the coverslip was screened for parasites, hence standardising the amount of faecal matter screened, at 40x magnification. If the sample was positive for coccidian parasites but the oocysts had not sporulated, approximately 2 mL of 2.2% potassium dichromate were added to the solution and the sample was left for 24 hours at room temperature to allow the oocysts to sporulate so that the genus of the parasite could be identified. Identification of the parasites at genus level was checked by sending some sample to Professor Stan Ball for confirmed identification.

Helminths parasites and ova were counted, and coccidian parasites were scored on a 0 to 5 scale following a methodology similar to Brawner III and colleagues (2000). Zero – no oocysts, 1 – 1 to 10 oocysts, 2 – 11 to 100 oocysts, 3- 101 to 1,000 oocysts, 4 – 1,001 to 10,000, 5 – over 10,000 oocysts. The oocysts were counted singly up to 500, after which the parasitemia was high enough to count the oocyst per field of view and compare the number found in each subsequent field of view so that the total number under the coverslip could be estimated correctly for high parasitaemia.

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Chapter 3

POPULATION HEALTH

3. POPULATION HEALTH

3.1 INTRODUCTION

Clinical haematology is one of the most informative procedures that can be used on wild birds to assess their health status (e.g. Artacho *et* al., 2007; Nadolski *et al.*, 2006; Campbell, 1995; Hawkey and Samour, 1988; Hawkey *et al.*, 1984). For example, values of leucocytes that are higher than normal (for the species) can indicate an infection, while a lower than normal leucocyte count can indicate immunosupression or some viral disease (Samour and Howlett, 2008) . From the differential leucocyte count it is possible to calculate the percentage of the five types of white blood cells found in birds (lymphocyte, heterophil, basophil, eosinophil, and monocyte), which will provide further information on the nature of an inflammatory response or stress (Samour and Howlett, 2008; Campbell and Ellis, 2007; Samour, 2006; Campbell, 1995). The absolute circulating value of each leukocyte type (lymphocytes, monocytes, and three granulocytes : heterophils, basophils and eosinophils) can be related to normal baseline values for the species or, in the absence of these, of a suitably comparable species. Normal WBC count reported for species similar in size to the house sparrow suggest a range between 4-10x10^9 /L of blood (Campbell and Ellis, 2007; Samour, 2006; Hauptmanová *et al.*, 2002).

Lymphocytes are typically round cells with heavily clumped chromatin in the nucleus and scant cytoplasm which gives a high nucleus to cytoplasm ratio (N:C) (Campbell and Ellis, 2007). The nucleus is often found off-centre in the cell. The size of lymphocytes can vary from as small as thrombocytes (from which can be distinguished from the lightly basophilic cytoplasm and from the less dense clumped chromatin in the nucleus), to as large as monocytes (from which they are distinguished by the higher N:C ratio, the more regular round shape, the lack of vacuoles in the cytoplasm, and the denser clumping of chromatin in the nucleus) (Campbell and Ellis, 2007). A higher than normal lymphocyte number is termed lymphocytosis and it is associated with infectious diseases and lymphocytic leukemia; a lower than normal lymphocyte number is termed lymphopenia and it associated with stress, uremia, and immunosuppressive conditions (Samour and

Howlett, 2008). There is a suggestion that lymphocyte numbers may vary with the circadian cycle, at least in nesting females, with higher levels in the night (Ots *et al.*, 1998), possibly due to more energy reserves being available for cell proliferation (Starck, 1996). The normal lymphocyte percentage varies between 30-60% (Campbell and Ellis, 2007) or 1-4x10^9 /L (Samour, 2006), although some authors have reported a percentage of 68.5% from healthy individuals (Hauptmanová *et al.*, 2002).

Monocytes are typically the largest leukocytes and they can vary in shape from round to amoeboid (Campbell and Ellis, 2007). The nucleus can also vary in shape from round to lobed, but it is generally much paler than that of lymphocytes, while the cytoplasm is abundant, pale blue in colour and it often contains vacuoles (Campbell and Ellis, 2007). Monocytes are long-lived phagocytic cells associated with defence against bacteria and infections and they often mould around other cells (Campbell, 1995). An increase in monocyte numbers, termed monocytosis, is associated with chronic inflammation (D' Aloia *et al.*, 1994). Normal values of monocytes in the blood range from 0-1% (Campbell and Ellis, 2007; Hauptmanová *et al.*, 2002) or 0-0.3x10^9/L (Samour, 2006).

Heterophils are the avian counterparts of mammalian neutrophils (Jain, 1993), and they are often the most abundant granulocytes in many avian species (Hawkey *et al.*, 1983). Heterophils are highly phagocytic and participate in the response to bacterial, viral, and parasitic infections (Campbell and Ellis, 2007). They tend to be round and their size is generally consistent within the same species (Campbell and Ellis, 2007). The cytoplasm of normal mature heterophils is colourless and it contains rod-shaped elongated granules (Campbell and Ellis, 2007). Higher than normal values of heterophils is termed heterophilia, and it associated with bacterial and fungal infection and general inflammatory response, as well as stress (Samour and Howlett, 2008; D'Aloia *et al.*, 1994); a lower than normal values of heterophils is referred to as heteropenia and it is associated with degenerative response to infection, bone marrow damage, viremia, and leukemia (Samour and Howlett, 2008). In reaction to pathogens, heterophils can undergo chemical and morphological alterations which are define "toxic changes", recorded on a scale of +1 to +4. Toxic changes generally involve increased cytoplasmic basophilia (toxic +1; toxic +2; toxic +3; toxic +4),

cytoplasmic vacuolation (toxic +2, toxic +3; toxic +4), degranulation (toxic +2; toxic +3; toxic +4) or granules that coalesce into one large granule (toxic +3, degeneration of cell nucleus (toxic +4). Their lysis during an inflammatory response, while aimed at destroying bacteria and other parasites, is also harmful to the host tissues (Parslow, 1994). The normal percentage of heterophils range between 20-60% of the leukocyte present (Campbell and Ellis, 2007) or 0.1-4.9x10^9 /L (Samour, 2006).

The heterophil to lymphocyte ratio (H/L ratio) is used as an indication of inflammation, as it increases during infection (Davis *et al.*, 2004), and as a measure of stress (Gross and Siegel, 1983) as it rises in response to mild to moderate long-term stressors (Ruiz *et al.*, 2002; Parga *et al.*, 2001; Vleck *et al.*, 2000; Maxwell, 1993; Averbeck, 1992) such as overcrowding (Gross and Siegel, 1983). In response to extreme stress, such as life-threatening situations, the H/L ratio decreases due to heteropenia (Maxwell, 1993). The H/L ratio, however, cannot be used as a predictive measure of the ability of an individual to mount an immune response (Davis *et al.*, 2008), because the ability of an individual to mount an immunological response against a specific pathogen cannot be generalised to all pathogens.

The H/L ratio is considered a reliable indicator of stress. Leukocyte numbers change more slowly (30 minutes to 20 hours) in response to stressors than do corticosterone (20-30 minutes) (Davis *et al.*, 2008; Davis, 2005; Romero and Reed, 2005; Maxwell, 1993; Dein, 1986; McFarne *et al.*, 1979), which remains elevated for shorter periods than the H/L ratio (McFarne *et al.*, 1979), indicating that the latter is a better indicator of chronic stress (Vleck *et* al., 2000; McFarne *et al.*, 1979). For example, H/L raises in response to heavy metal pollution. Blanco and colleagues (Blanco *et al.*, 2004) found an increased H/L ratio in relation to heavy metal pollution in red kites (*Milvus milvus*), and Eeva and colleagues (Eeva *et al.*, 2005) found that the H/L ratio was a better indicator of cadmium and nickel pollution stress than corticosteroids in nestling pied flycatchers. The H/L ratio of nestlings from polluted areas was higher than in non-polluted ones, and H/L ratio was also inversely correlated to the probability of fledging (Eeva *et al.*, 2005). However, the effect of invertebrate availability cannot be separated from pollution, since a previous study had shown lower invertebrate abundance in the same sites (Eeva *et al.*, 1997). The

increased H/L ratio in polluted environments may therefore also be explained by food deprivation, which causes increased level of corticosterone (Vleck *et* al., 2000), which in turn raises the H/L ratio (Maxwell, 1993; Gross *et al.*, 1980).

Studies have shown that the H/L ratio can indicate stress of breeding. In an experiment using great tits Hõrak and colleagues showed that birds with enlarged broods in rural areas had higher H/L ratio than parents with control or reduced broods (Hõrak *et al.*, 1998). There is also a suggestion that the H/L ratio may be higher in females than males especially during the breeding season (Kilgas *et al.*, 2006; Ots *et al.*, 1998), although this was not found in all studies (Vleck *et al.*, 2000) probably because the H/L ratio is one of the most variable haematological traits within the same population (Ots *et al.*, 1998).

Eosinophils are similar in size to heterophils, but in contrast to the latter the cytoplasm stains clear blue and the cytoplasmic granules are typically round. An increase in eosinophils, termed eosinophilia, is associated with parasite infection (Maxwell, 1987), while a decrease in eosinophils, termed eosinopenia, is associated with stress (Samour and Howlett, 2008), but no changes have been recorded in chronic inflammations (D-Aloia *et al.*, 1994). The normal range of eosinophils is 0-1% (Campbell and Ellis, 2007) or 0-0.3x10^9 /L (Samour, 2006), but percentage of 5.6% from healthy individuals has also been reported (Hauptmanová *et al.*, 2002).

Basophils contain deeply metachromic granules that often obscure the nucleus, which is usually non-lobed (Campbell and Ellis, 2007). The function of basophils is still unclear but they are thought to take part in acute inflammatory responses (Campbell and Ellis, 2007), although basophilia has been reported in cases of chronic inflammation (D'Aloia *et al.*, 1994) and prolongued period of stress (Maxwell *et al.*, 1990). The normal range of basophils varies between 0-5% (Campbell and Ellis, 2007) or 0-0.4x10^9 /L (Samour, 2006), but a slightly higher percentage of 5.6% from healthy individuals has also been reported (Hauptmanová *et al.*, 2002).

Total leukocyte count can change with age, season and moult. The predominant type of leukocyte varies with season and reproductive status (Abelenda *et al.*, 1993; Driver, 1981), age (Puerta *et* al., 1992; Alonso *et* al., 1991; Fairbrother and

O'Loughling, 1990; Puerta *et* al., 1990; Puerta *et al.*, 1989), and moult (Nava *et al.*, 2001; Driver, 1981). Some authors have found differences in sexes with females having higher heterophil counts than males (Kilgas *et al.*, 2006; Ots *et al.*, 1998), while other authors have found no differences (Davis *et al.*, 2004). Juvenile birds tend to have higher total leukocyte count than adults (Davis *et al.*, 2004; Puerta *et al.*, 1995), and have higher lymphocyte, monocytes and basophil counts (Davis *et al.*, 2004; Nava *et al.*, 2001). Adults generally have higher heterophil counts than juveniles (Alondo *et al.*, 1991; Hawkey *et al.*, 1983) but not in all species (Fairbrother and O'Loughling, 1990). Moulting birds have been reported to have higher eosinophils than non-moulting birds (Davis *et al.*, 2004), and birds of both sexes can suffer from lymphopenia associated with the high stress of reproduction (Hõrak *et al.*, 1998).

The number of circulating erythrocytes (RBC) , quantity of haemoglobin (Hb), the volume of the red blood cells [called Packed Cell Volume or haematocrit (PCV or Hct)], and the relationship between them (MCV, MCHC, MCH) provide and indication of the capacity of oxygen transportation of the animal (Sturkie and Giminger, 1976). Polycythemia (the increase in PCV/Hct and RBC) may be a reaction to hypoxia, chronic respiratory diseases, or dehydration (shown when PCV is greater than 56%) (Samour, 2006). Decreased PCV (<35% (Campbell, 1995) and RBC [normal range : 2.5-5x10^{12}/L (Campbell and Ellis, 2007; Nadolski *et al.*, 2006; Parga *et al.*, 2001; Davey *et* al., 2000; Kostelecka-Myrcha, 1997; Breuer *et al.*, 1995)] indicate anaemia that can be caused by haemorrhage due to trauma, ectoparasitism, endoparasitism, erythrocyte destruction due to haemoparasites, decreased erythrocyte production due to nutritional deficiencies, chronic infection, and toxicosis, among others (Samour, 2006). Normal range of Hb is 11-18 g/L (Bańbura *et* al., 2007; Nadolski *et al.*, 2006; Parga *et al.*, 2001; Davey *et al.*, 2000; Arnold *et* al., 1999; Kostelecka-Myrcha, 1997; Breuer *et* al., 1995; Puerta *et al.*, 1995; Ruiz *et al.*, 1995; Palomeque *et al.*, 1980), and values below 11g/L are indicative of anaemia (Samour, 2006).

Several studies have found a link between parasite infection and haematocrit and/or haemoglobin (Dudaniec *et al.*, 2006; Słomczyński *et al.,* 2006; Booth and Elliott, 2003; Potti *et al.*, 1999; Hurrez-Boussès *et al.*, 1997; Shutler *et al.*, 1996; Whitworth

and Bennett, 1992; Chapman and George, 1991; Møller, 1991; Harrison and Harrison, 1986) although some studies have found no such correlation (Johnson and Albrecht, 1993). Hb values should always be reported alongside PCV values, because birds that are affected by haematophagous parasites may replenish lost erythrocyte through fast erythropoiesis (Campbell, 1995; Dein, 1986) but reticulocytes will not carry as much haemoglobin as mature erythrocytes (Jain, 1993). A study on house wrens (*Troglodytes aedon*) found that while nestling haematocrit was not affected by blow fly larvae nest infestation, Hb of parasitised chicks was 25% lower than non-parasitised nestlings (O'Brien *et al.*, 2001). In migratory birds the relationship between PCV and parasites was not apparent (Jenni *et al.*, 2006).

Haematocrit shows seasonal variation. Several studies have found that PCV is higher in winter than in summer (Kostelecka-Myrcha, 1997; Swanson, 1991; Clemens, 1990; Swanson, 1990; Rehder and Bird, 1983; deGraw *et al.*, 1979; Carey and Morton, 1976), and that total erythrocyte count was higher and MCV lower in winter than in summer (Kostelecka-Myrcha, 1997; Breuer *et al.*, 1995; Ruiz, *et al.*, 1995). Some authors have found that Hb values were at the highest in winter and the lowest in spring, with a gradual increase in summer and autumn (Kostelecka-Myrcha, 1997; Puerta *et al.*, 1995) but other studies have found no seasonal differences in PCV or Hb values (Breuer *et* al., 1995; Ruiz *et* al., 1995; Rooke *et al.*, 1986). Changes in haematocrit can be caused by a combination of factors including reproductive status, thermoregulation (Carey and Morton, 1976; Sealander, 1962), moult and photoperiod (Rehder *et al.*, 1982).

Erythropoiesis can be exercise-induced in response to hypoxia or increased workload (Hõrak *et al.*, 1998; Clemens, 1990; Jaeger and McGrath, 1974). In adult Great tits haematocrit increased in response to brood enlargement, suggesting a physiological adaptation to higher oxygen demand of the individual (Hõrak *et al.*, 1998). In the barn swallow, *Hirundo rustica*, males with an experimentally elongated tail had higher haematocrits, possibly in response to a higher energy demand imposed by flying with a longer tail (Saino *et al.*, 1997). Migratory birds have higher haematocrits during migration (Piersma *et* al., 1996; Prats *et* al., 1996; Barlein and Totzke, 1992) suggesting the need to improve oxygen carrying capacity and tissue

oxygenation during that phase. However, not all migratory birds show an increase in haematocrit in response to physical activity. For example, in a study on passerine and waders, Jenni and colleagues showed that birds decrease their PCV to decrease blood viscosity, sometimes resulting in anaemia especially if the bird finishes its fat reserve and starts breaking down proteins (Jenni *et al.*, 2006). Fasting is not always associated with decreased haematocrit. Adélie penguins (*Pygoscelis adeliae*) showed increased haematocrit and Hb concentration, presumably from dehydration, while fasting as part of their normal breeding biology (Vleck *et al.*, 2000).

Haematocrit has been reported to change with reproductive status (Williams *et al*, 2004, Morton, 1994). The on-set of the breeding season causes hormonal changes that affect the haematocrit level since androgens stimulate and oestrogen inhibit erythrocyte synthesis (Rehder and Bird, 1983). Reproducing males tend to have higher haematocrit than reproductive females (Sturkie and Giminger, 1976), possibly through a testosterone-mediated mechanism (Buttemer And Astheimer, 2000; Rehder *et al.*, 1982). In an experiment on house sparrows implanted with testosterone, Puerta and colleagues (Puerta *et al.*, 1995) showed that testosterone increased levels of circulating erythrocytes and Hb. The role of steroids, and testosterone in particular, on haematocrit values remains unclear (Alonso-Alvarez *et al.*, 2002), but corticosterone produced after the stimulus of a stressor seems to depress the haematocrit (Lynn *et al.*, 2003).

Breeding imposes increased metabolic demands on parents of altricial species (Walsberg, 1983). Egg-production increases energy demand by 13-41% of the BMR (Carey, 1996), and feeding nestling is probably the most energetically demanding process (Daan *et* al., 1990) since nestling metabolise 2-2.5 times the BMR per day (Drent *et* al., 1992; Weathers, 1992). Breeding therefore requires a higher metabolic consumption and associated higher oxygen requirement for the parents, and in species with protracted breeding cycles there may be more time for an adjustment in the blood oxygen transport capability (Davey *et* al., 2000), and this may be the case in the house sparrow. This theory found support in a study (Davey *et* al., 2000) which found that PCV, RBC, and Hb increased with the progression of the breeding season, with an associated decrease in body mass. Higher metabolic rates and oxygen

demands in animals with a smaller body may explain why they tend to have larger erythrocytes than larger individuals (Hartman and Lessler, 1963).

Haematocrit and Hb quantity have been shown to increase with age of the bird (Simmons and Lill, 2006; Shapiro *et al.*, 1999; Kostelecka-Myrcha *et* al., 1997; Merino and Barbosa, 1997; Puerta *et al.*, 1995), although this was not the case in all studies (Ots *et al.*, 1998). MCV and MCH decrease with age while MCHC increase linearly throughout development until adult age (Simmons and Lill, 2006; Kostelecka-Myrcha *et al.,* 1972), possibly in order to obtain a balance between oxygen transportation and blood viscosity (Scmidt-Nielsen, 1995; Wells and Baldwin, 1990).

There is contrasting evidence of difference in haematocrit between males and females (Morton, 1994; Carey and Morton, 1976) with some authors reporting no difference between the sexes (Fair *et* al, 2007; Jenni *et al.*, 2006; Davey *et al.*, 2000; Saino *et al.*, 1997), and others suggesting that females have higher haematocrit than males (Kilgas *et al.*, 2006; Ots *et al.*, 1998) possibly linked to higher workload of the females during the breeding season (Kilgas *et al.*, 2006).

Several studies on wild birds have assumed a link between haematocrit and body condition (e.g. Svensson and Merilä, 1996; Johnson and Albrecht, 1993; Carpenter, 1975), although some authors have not found such a link in either adults or juveniles (Villegas *et* al., 2002; Dawson and Bortolotti, 1997a;b). A cross-fostering experiment on barn swallow (*Hirundo rustica*) in Spain showed that body condition was significantly related to haematocrit in nestlings, but that this relationship was weak due to high variability associated with a strong environmental component (Cuervo *et al.*, 2007). The same authors found no heritability for this trait (Cuervo *et al.*, 2007), a result in agreement with a study on Pied flycatchers (*Ficedula hypoleuca*) (Potti *et al.*, 1999).

Fibrinogen is a plasma protein involved in blood clotting, and its normal values range 0.9-4.0 g/L (Samour, 2006). Raised value of this blood component may indicate an ongoing infection, while a lower than normal value can indicate liver failure (Samour and Howlett, 2008). Fibrinogen has not been reported to vary with

age, sex, exercise, or repeated bleeding but it can be altered by moderate inflammatory states, although the increase in fibrinogen levels is not always in proportion to the severity of the disease process (Jain, 1993).

Normal values for house sparrows are provided only by a few studies based on few birds sampled in a limited time window (Puerta *et* al., 1995; Gavett and Wakeley, 1986; Palomeque *et* al, 1980; Baumann and Baumann, 1977; Nice *et al.*, 1935), but additional normal values can be established using other species of comparable size and ecology (for example finches, which are similar in size and also exhibit gregarious behaviour, and they often feed on the ground, like house sparrows). The average haematocrit for house sparrows vary between 39.4 and 53.1% (Gavett and Wakeley, 1986; Palomeque *et al.* 1980; Baumann and Baumann, 1977), haemoglobin level varied 110-140g/L based on a study of 69 house sparrow in June and September in Spain (Puerta *et al.*, 1995) but one record of 173 g/L was also reported (Palomeque *et al.*, 1980). Total leucocyte counts for house sparrow ranged from 6.500 to 29.500 per mm^3 (Nava *et al.*, 2001; Puerta *et al.*, 1995) but the authors had not checked for presence of infectious agents that could have raised the total count.

Parasite ecology and pathogenicity were described in Chapter 1.

3.2 AIM

The aims of this chapter are:

1. To indicate basal haematological values for house sparrows.
2. To investigate variation of haematological values between seasons, age, sex and condition.
3. To give an overview of the prevalence of bacteria, haemoparasites, and gastrointenstinal-parasites found during the study.
4. To investigate, using standard clinical tests, whether there was a physiological reaction to the pathogens (and hence assess their potential pathogenicity to the host).

3.3 METHODOLOGY

Samples of faecal matter from birds and bird tables were collected as described in Chapter 2, and blood as described in section 2.2.2. Faecal and blood sampled were tested for parasites as described in section 2.3.3 and 2.3.1, respectively.

The basal haematological values found in the literature are often based on studies that have not specified the presence or absence of parasites, and therefore they are potentially including biased values. In order to minimise this type of error, basal haematological values in this study were based on individuals that had tested negative for any bacterial or parasitic infection considered in this study.

The physiological changes associated with presence of parasites were investigated by relating results on *Plasmodium* spp count, *Atoxoplasma* spp count, and *Isospora* spp count, separately, with total leucocytes count, differential count, total erythrocyte count, haemoglobin, packed cell volume, MCH, MCHC, MCH, and fibrinogen.

Condition was calculated as the residuals of the linear relationship between body mass and tarsus length. Breeding status was considered positive when a female bird had a brood patch, and a male bird a cloacal protuberance, and birds that were considered non-breeding were those that did not show a brood patch or a cloacal protuberance in the months of the breeding season (April to August) and that were not moulting. Moulting period was considered, for adults, between July and end of September, and they were considered as not moulting if during that period they were not showing signs of active remiges moult nor breeding (since house sparrows do not usually initiate moult until after breeding is complete (Anderson, 2006). In analyses that did not differentiate between ages or sexes, the data had come from pooled sample of both ages and sexes.

3.3.1 Sample size

Birds that tested negative to the pathogen considered in this study

Table. 3.1. Sample-size of birds that tested negative for pathogens considered in this study. ♀= Females. ♂= Males. U= Unsexed.

Variable	Adults	Juveniles	Total
Total Leucocytes	63 (29♀; 34♂)	57 (13♀; 17♂; 27U)	120
Lymphocytes	90 (40♀; 50♂)	115 (17♀; 45♂; 53 U)	205
Heterophils	90 (40♀; 50♂)	115 (17♀; 45♂; 53 U)	205
Basophils	90 (40♀; 50♂)	115 (17♀; 45♂; 53 U)	205
Eosinophils	90 (40♀; 50♂)	115 (17♀; 45♂; 53 U)	205
Monocytes	90 (40♀; 50♂)	115 (17♀; 45♂; 53 U)	205
Total erythrocytes	60 (27♀; 33♂)	54 (13♀; 25♂; 16 U)	114
Haemoglobin	65 (29♀; 36♂)	88 (16♀; 36♂; 36 U)	153
Packed Cell Volume	41 (16♀; 25♂)	25 (8♀; 13♂; 4 U)	66
Mean Corpuscular Volume	37 (16♀; 21♂)	24 (8♀; 12♂; 4 U)	61
Mean Corpuscular Haemoglobin	50 (22♀; 28♂)	38 (10♀; 19♂; 9 U)	88
Mean Corpuscular Haemoglobin Concentration	40 (16♀; 24♂)	20 (7♀; 10♂; 3 U)	60
Fibrinogen	34 (14♀; 20♂)	24 (9♀; 13♂; 2 U)	58
Condition	89 (40♀; 49♂)	114 (17♀; 44♂; 53 U)	203
Breeding Status (Y= Breeding; N= Not-Breeding)	29 (20Y; 14 N)	N/A	29
Moult (M=Moulting) (C=Not-moulting)	15 (3M ; 12 C)	113 (61 M; 52 C)	128

Table. 3.2. Sample size across seasons. Values from birds tested negative for any pathogen.

Variable	Winter	Spring	Summer	Autumn
Total Leucocytes	21 (22 Ad;0 J)	27(14 Ad;13 J)	47 (3 Ad; 44 J)	25 (25 Ad;0J)
Lymphocytes	22 (22 Ad; 0J)	78 (28 Ad; 50J)	71 (6 Ad; 65 J)	34(34 Ad; 0J)
Heterophils	22 (22 Ad; 0J)	78 (28 Ad; 50J)	71 (6 Ad; 65 J)	34(34 Ad; 0J)
Basophils	22 (22 Ad; 0J)	78 (28 Ad; 50J)	71 (6 Ad; 65 J)	34(34 Ad; 0J)
Eosinophils	22 (22 Ad; 0J)	78 (28 Ad; 50J)	71 (6 Ad; 65 J)	34(34 Ad; 0J)
Monocytes	22 (22 Ad; 0J)	78 (28 Ad; 50J)	71 (6 Ad; 65 J)	34(34 Ad; 0J)
Total erythrocytes	20 (20 Ad; 0J)	24 (14 Ad; 14J)	47 (3 Ad; 44J)	23(23 Ad; 0J)
Haemoglobin	19 (19 Ad; 0J)	53 (19 Ad; 34J)	58 (4 Ad; 54J)	23(23 Ad; 0J)
Packed Cell Volume	17 (17 Ad; 0J)	10 (8 Ad; 2J)	24(1 Ad; 23J)	15(15 Ad; 0J)
Mean Corpuscular Volume	16 (16 Ad; 0J)	10(8 Ad; 2J)	22 (0 Ad; 22J)	13(13 Ad; 0J)
Mean Corpuscular Haemoglobin	18 (18 Ad; 0J)	17 (12 Ad; 5J)	35(2 Ad; 33J)	18(18 Ad; 0J)
Mean Corpuscular Haemoglobin Concentration	17(17 Ad; 0J)	10 (8 Ad; 2J)	19 (1 Ad; 18J)	14(14 Ad; 0J)
Fibrinogen	16 (16 Ad; 0J)	7 (6 Ad; 1J)	23 (0 Ad; 23J)	12(12 Ad; 0J)
Condition	22 (22 Ad; 0J)	78 (27 Ad; 51J)	70 (6 Ad; 64J)	34(34 Ad; 0J)
Breeding Status (Y= Breeding; N= Not-Breeding)	0	23 Y; 4 N	2 Y; 0 N	0
Moult (M=Moulting) (C=Not-moulting)	0	7 Y (0 Ad; 7J) 52N(11Ad;41J)	57Y(3Ad;54J) 12N(1Ad;11J)	0

Infected birds

Table 3.3. Sample size of haematological values of birds infected with either *Plasmodium*, *Atoxoplasma*, or *Isospora*. Prev= Used to calculate values related to prevalence of infection; Int= Used to calculate values related to intensity of infection.

Variable	*Plasmodium*	*Atoxoplasma*	*Isospora*
Total Leucocytes	166 Prev; 44 Int	179 Prev ; 54 Int	120 Prev;28 Int
Lymphocytes	264 Prev ; 57 Int	291 Prev ; 84 Int	115 Prev;39 Int
Heterophils	264 Prev ; 57 Int	291 Prev ; 84 Int	115 Prev;39Int
Basophils	264 Prev ; 57 Int	291 Prev ; 84 Int	115 Prev;39 Int
Eosinophils	264 Prev ; 57 Int	291 Prev ; 84 Int	115 Prev;39 Int
Monocytes	264 Prev ; 57 Int	291 Prev ; 84 Int	115 Prev;39 Int
Total erythrocytes	158 Prev ; 42 Int	169 Prev ; 52 Int	114 Prev;26 Int
Haemoglobin	200 Prev ; 45 Int	220 Prev ; 63 Int	153 Prev;31 Int
Packed Cell Volume	96 Prev ; 29 Int	99 Prev ; 32 Int	66 Prev; 17 Int
Mean Corpuscular Volume	90 Prev ; 28 Int	92 Prev ; 30 Int	61 Prev; 17 Int
Mean Corpuscular Haemoglobin	127 Prev ; 37 Int	132 Prev ; 42 Int	88 Prev; 20 Int
Mean Corpuscular Haemoglobin Concentration	88 Prev ; 27 Int	88 Prev ; 27 Int	60 Prev; 16 Int

3.3.2 Statistical analyses

The basal haematological values were calculated using the values of those individuals that were negative for any parasites or pathogenic bacteria. Only three parasites (*Plasmodium* spp., *Atoxoplasma* spp., and *Isospora* spp.) were considered for the analyses of the haematological reactions due to the small sample size of the other parasites.

The intensity and prevalence of the parasites were analysed separately per each genus. Prevalence indicated the percentage of birds infected, while intensity indicated the level of the infection of the parasitised individuals. To investigate the physiological response at the haematological level to each parasite genus, only data for birds with only that specific parasite were used to avoid the bias of a synergistic effect of more than one parasite genus on the host.

Samples coming from the same sampling unit are not independent (Crawley, 2007). Birds coming from the same sites and multiple samples from the same individual cannot be considered independent but, rather, pseudoreplicates. In order to account for this, Mixed Effect Models were applied on only the first sample taken from each bird , and site was used as random effect. The relationship between the variables was checked visually for non-linearity so Linear Mixed Effect Models were used instead of Generalised Mixed Effect Models.
All analyses were conducted in R 2.11.1 (R Development Core team, 2010) using Tinn-R R (Development Core team, 2010) as text interface.

Basal haematological values analyses

The mean and standard deviation of each haematological value were calculated using data from birds that had tested negative for any pathogens included in this study. The absolute value of each leucocyte type was obtained by multiplying its mean percentage by the mean total leucocyte value, and standard deviation was also calculated for each mean.

For all other analyses

Linear Mixed Effect Models were built using the lmer function in R. Each of the haematological values was used in turn as the response variable, and either season, age, sex, moult or condition as the explanatory variable (fixed effect). Site was used as random effect to account for spatial pseudoreplication, and only the first observation from each bird was analysed to avoid pseudoreplication at the individual level. Only birds that had tested negative for pathogens included in this study were used. The significance of each explanatory variable was calculated by comparing the model with the variable to a model without the variable using the likelihood ratio test, with maximum likelihood, as suggested by Douglas Bates (2006). This method

utilises a chi-square distribution, therefore the chi-square value, p-value, and degrees of freedom (df) for each test were reported.

Binomial error structure was used for analyses with lymphocytes, heterophils, monocytes, basophils, eosinophils, and heterophil/lymphocyte ratio as response variable. These variables are proportions because they can only range from 0 to 100. Overdispersion was checked using the "summary(model)@sigma" function, and if the value was much greater than 1, a quasi-binomial distribution was used instead. Quasi-poisson error structure was used with haemoglobin as response variable because it contained positive integer values, but the data were overdispersed hence the poisson distribution would not have been appropriate. Gamma error structure was used with total leucocyte count, fibrinogen quantity, MCH, or MCHC as response variable, because they were continuous positive variables that did not contain zeros, but they were skewed and not following the normal distribution. Total erythrocyte (RBC) , Packed Cell Volume, and MCV followed a normal distribution.

The prevalence and intensity of each parasite across site was tested using Generalised Linear Models instead of Linear Mixed Effect Models, because by using site as the explanatory variable the pseudoreplication at the site level was already accounted for. Binomial error structure was specified for analyses with parasite prevalence as response variable. Quasi-poisson error structure was used with parasite intensity as response variable, because it could potentially contain any values from zero to $+\infty$, but the data were overdispersed, hence a poisson distribution would have not been appropriate.

3.4 RESULTS

3.4.1 Basal haematological values

Table 3.4. Haematological values of sparrows that tested negative for pathogens considered in this study.

Haematological value	Mean ± s.d.	Range	Sample size
Total Leucocytes (10^9/L)	13.11 ± 7.74	3.05-46.7	n = 120
Lymphocytes (%)	49.92 ± 15.98	10-90	n = 205
Heterophils (%)	21.37 ± 13.92	2-74	n = 205
Basophils (%)	9.6 ± 9.04	0-60	n = 205
Eosinophils (%)	2.16 ± 2.76	1-24	n = 205
Monocytes (%)	6.92 ± 9.78	0-48	n = 205
Total erythrocytes (10^{12}/L)	4.09 ± 1.62	1.96-19.1	n = 114
Haemoglobin (g/L)	187.59 ± 33.68	107-254	n = 153
Packed cell volume (%)	48.9 ± 7.58	27.83-75.0	n = 66
Mean corpuscular volume (fL)	118.1 ±25.25	39.27-189.1	n = 61
Mean corpuscular haemoglobin (pg)	486.88 ± 123.6	109.4-837.9	n= 88
Mean corpuscular haemoglobin concentration (gm/dl)	41.07 ± 9.47	25.3-86.6	n = 60
Fibrinogen (g/L)	2.42 ± 2.45	0.28-14.29	n = 58

Table. 3.5. Absolute leucocyte values of sparrows that tested negative for pathogens considered in this study

Haematological value	Mean ± s.d.	Range
Lymphocyte (10^9/L)	6.78 ± 4.05	0.56 – 18.36
Heterophils (10^9/L)	2.69 ± 3.46	0.14 – 23.35
Basophils (10^9/L)	1.29 ± 1.22	0 – 8.4
Eosinophils (10^9/L)	0.3 ± 0.45	0 – 2.93
Monocytes (10^9/L)	2.04 ± 1.84	0.09 – 10.9

3.4.2 Haematological changes between years, seasons, ages, sexes, moult and condition of non-parasitised individuals

Variation between years

Table 3.6. Variation of haematological parameters across the study period. WBC=White Blood Cells; H/L=Heterophil/Lymphocyte ratio; RBC= Red Blood Cells; Hb= Haemoglobin; PCV=Packed Cell Volume; MCH=Mean Corpuscular Haemoglobin; Fib=Fibrinogen; N.S.=Not Significant; *= $p<0.05$; ** = $p<0.01$; *** = $p<0.0001$.

	WBC	**Lymphocyte**	**Heterophil**	**H/L**	**Eosinophil**	**Basophil**
Year	N.S.	N.S.	***	*	***	***
	Monocyte	**RBC**	**Hb**	**PCV**	**MCH**	**Fibrinogen**
Year	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

Total leukocyte count did not vary across years (χ^2=0.18, p=0.664, df=1). Lymphocyte numbers (χ^2=3.09, p=0.07, df=1) and monocyte numbers (χ^2=7.65, p=0.0056, df=1) did not vary between years but heterophils did (χ^2=10.39, p=0.0012, df=1). The heterophil/lymphocyte ratio (H/L ratio) also differed between years (χ^2=5.36, p=0.02, df=1), as did eosinophils (χ^2=8.74, p=0.0031, df=1), and basophils (χ^2=13.43, p=0.00024, df=1). Total erythrocyte count did not vary across years (χ^2=0.08, p=0.77, df=1), neither did haemoglobin (χ^2=1.23, p=0.27, df=1), PCV (χ^2=1.26, p=0.26, df=1), MCH (χ^2=1.69, p=0.1923, df=1), nor Fibrinogen (χ^2=3.70, p=0.233, df=1).

Variation between seasons

Table 3.7. Variation of haematological parameters across seasons. WBC=White Blood Cells; Lym=Lymphocytes; Het=Heterophils; H/L=Heterophil/Lymphocyte ratio; Eos=Eosinophils; Bas=Basophils; Mon=Monocytes; RBC= Red Blood Cells; Hb= Haemoglobin; PCV=Packed Cell Volume; MCH=Mean Corpuscular Haemoglobin; Fib=Fibrinogen; N.S.=Not Significant; *= $p<0.05$; ** = $p<0.01$; *** = $p<0.0001$.

	WBC	**Lymp**	**Hete**	**H/L**	**Eosi**	**Baso**
Overall	***	N.S.	***	**	N.S.	N.S.
Summer Spring	***	N.S.	N.S.	N.S.	N.S.	N.S.
Summer Autumn	N.S.	*	N.S.	N.S.	***	N.S.
Summer Winter	N.S.	*	N.S.	*	*	N.S.
Spring Autumn	N.S.	N.S.	N.S.	N.S.	**	N.S.
Spring Winter	N.S.	N.S.	N.S.	N.S.		N.S.
Autumn Winter	N.S.	N.S.	*	N.S.	*	*
	Mono	**RBC**	**Hb**	**PCV**	**MCH**	**Fibr**
Overall	N.S.	N.S.	***	**	N.S.	N.S.
Summer Spring	N.S.	*	***	*	**	N.S.
Summer Autumn	N.S.	N.S.	*	N.S.	*	N.S.
Summer Winter	**	N.S.	N.S.	**	N.S.	*
Spring Autumn	N.S.	N.S.	**	*	***	N.S.
Spring Winter	N.S.	N.S.	N.S.	***	N.S.	***
Autumn Winter	*	N.S.	***	***	N.S.	N.S.

Total leukocyte count was significantly different across seasons (χ^2 =26.53, p=7.37e-06, df=3). It was higher in summer than in spring (χ^2=11.14, p=0.00084, df=1) but it was not significantly different between summer and autumn (χ^2= 1.47, p=0.27, df=1) nor summer and winter (χ^2=1.90, p=0.16, df=1), nor spring and autumn (χ^2= 1.01, p=0.3, df=1) , nor spring and winter (χ^2=0.65, p=0.42, df=1), nor winter and autumn (χ^2=0.68, p=0.41, df=1).

Lymphocyte count did not differ across seasons overall (χ^2= 2.42, p=0.48, df=3). However, it was higher in summer than in autumn (χ^2=4.48, p=0.034, df=1) and higher in summer than in winter (χ^2=6.16, p=0.013, df=1) but it was not significantly different between spring and autumn (χ^2=0.31, p=0.58, df=1), spring and winter (χ^2=0.05, p=0.81, df=1) winter and autumn (χ^2=3.59, p=0.053, df=1), nor summer and spring (χ^2=2.2, p=0.13, df=1).

Heterophil count varied across seasons (χ^2 =16.89, p=0.0007, df=3), but the only significant difference was a lower count in winter than in autumn (χ^2 =5.79, p=0.016, df=1). Heterophil count did not vary between summer and spring (χ^2 =2.62, p=0.11, df=1), summer and autumn (χ^2 =0.97, p=0.32, df=1), summer and winter (χ^2 =1.25, p=0.26 , df=1), spring and autumn (χ^2 =0.36, p=0.55, df=1), or spring and winter (χ^2 =0.37, p=0.54, df=1).

The H/L ratio differed between seasons (χ^2 =12.39, p= 0.006, df=3) but the only significant difference was a higher H/L ratio in summer than in winter (χ^2 =4.23, p=0.04, df=1), but none of the other season by season pairwise comparison was significant: summer and spring (χ^2 = 2.70, p=0.10, df=1), summer and autumn (χ^2 =2.21, p=0.13, df=1), spring and autumn (χ^2 =1.89, p=0.17, df=1), spring and winter (χ^2 =1.91, p=0.16, df=1), and winter and autumn (χ^2 =1.99, p=0.15, df=1).

Monocyte count was not different between seasons overall (χ^2 =6.9, p=0.075, df=3). There was no difference between summer and spring (χ^2=1.02, p=0.31, df=1), nor summer and autumn (χ^2=0.65, p=0.41, df=1), but monocyte count was lower in summer than in winter (χ^2=7.02, p=0.008, n=205). No difference was present between spring and autumn (χ^2=3.78, p=0.051, df=1), and spring and winter

(χ^2=2.77, p=0.09, df=1), but monocyte count was lower in winter than in autumn (t=4.82, p=0.028, df=1).

Eosinophil count did not differ across seasons overall (χ^2 =4.04, p=0.257, df=3). There was no significant difference between summer and spring (χ^2=0.31, p=0.57, df=1), but count was significantly higher in summer than in autumn (χ^2=20.72, p=5.299e-06, df=1), higher in summer than in winter (χ^2=3.98, p=0.046, df=1), higher in spring than in autumn (χ^2=7.54, p=0.006, df=1), and higher in winter than in autumn (χ^2=6.2, p=0.012, df=1), but it was not significantly different between spring and winter (χ^2=0.73, p=0.39, df=1).

Basophil count did not differ across seasons overall (χ^2 =39.53, p=1.338e^{-08}, df=3). The only significant season pairwise difference was that counts in winter were higher than in autumn (χ^2=5.47, p=0.019, df=1), but there was no difference between summer and spring (χ^2=1.94, p=0.16, df=1), summer and autumn (χ^2=2.12, p=0.15, df=1), summer and winter (χ^2=2.15, p=0.14, df=1), spring and autumn (χ^2=0.19, p=0.66, df=1), and spring and winter (χ^2=0.2, p=0.65, fd=1).

Total erythrocyte did not differ overall across seasons (χ^2 =5.57, p=0.134, df=3), but it was higher in spring than in summer (χ^2=4.96, p=0.02, df=1). None of the other season pairwise differences was significant: summer and autumn (χ^2=1.52, p=0.21, df=1), summer and winter (χ^2=2.66, p=0.11, df=1), spring and autumn (χ^2=0.74, p=0.39, df=1), spring and winter (χ^2=0.7, p=0.4, df=1), and winter and autumn (χ^2=0.6, p=0.44, df=1).

Haemoglobin varied significantly across seasons (χ^2 =166.04, p<2.2e-16, df=3). It was lower in autumn than in spring (χ^2=3.10, p= 0.0019, df=1) and lower in autumn than in winter (χ^2=12.32, p<2.2e-16, df=1) but higher in summer than in autumn (χ^2=34.31, p=0.034, df=1). Haemoglobin was lower in summer than in spring (χ^2=103.66, p<2.2e-16, df=1) and higher in summer than in autumn (χ^2=34.31, p=4.86e-09, df=1) but not significantly different between summer and winter (χ^2=2.08, p=0.15, df=1) or between spring and winter (χ^2=2.70, p=0.11, df=1).

Packed Cell Volume varied between seasons (χ^2 =12.2, p=0.006, df=3). It was lower in summer than in spring (χ^2= 6.04, p=0.013, df=1), lower in summer than in autumn (χ^2=5.30, p=0.021, df=1), and lower in summer than in winter (χ^2=10.08, p=0.0014, df=1). It was higher in spring than in autumn (χ^2=39.21, p<2.2e-16, df=1), in spring than in winter (χ^2=27.27, p<2.2e-16, df=1), and higher in winter than in autumn (χ^2=31.02, p<2.2e-16, df=1).

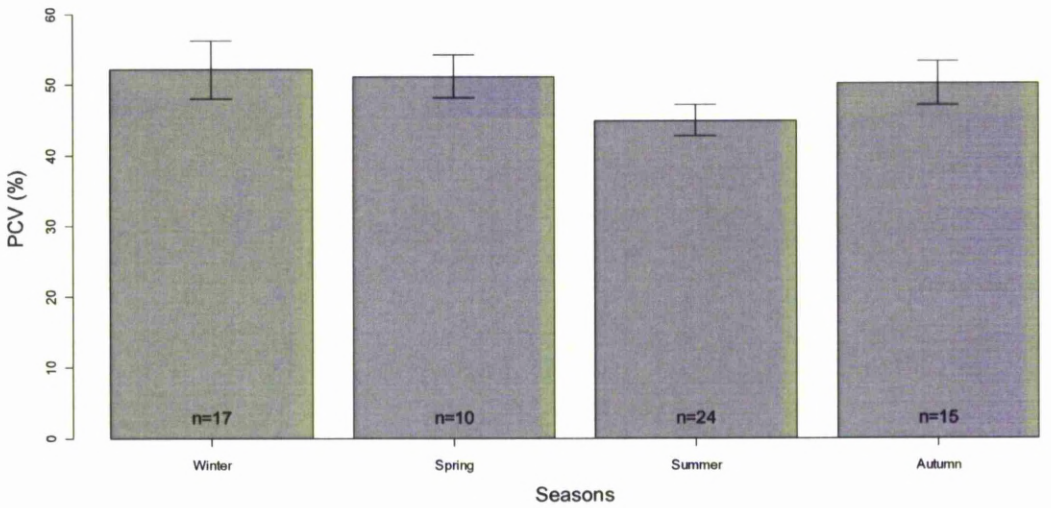

Fig. 3.1. Variation of Packed Cell Volume across seasons. The error bars indicate ± 1 s.e.

Overall difference of MCH between seasons was almost significant (χ^2 =7.59, p= 0.055, df=3). MCH in spring was significantly higher than in summer (χ^2=7.27, p=0.006, df=1), but lower in spring than in autumn (χ^2=14.31, p=0.0001, df=1), and lower in summer than in autumn (χ^2=6.09, p=0.013, df=1), but none of the other season pairwise differences was significant: summer and winter (χ^2=0.15, p=0.69, df=1), spring and winter (χ^2=0.21, p=0.65 , df=1), and winter and autumn (χ^2=0.92, p=0.33, df=1).

Fibrinogen did not vary across seasons (χ^2=4.27, p=0.23, df=3). There was no difference between summer and spring (χ^2=3.17, p=0.07, df=1), summer and autumn (χ^2=0.15, p=0.92, df=1), spring and autumn (χ^2=0.68, p=0.41, df=1), nor between winter and autumn (χ^2=0.57, p=0.45, df=1). However, fibrinogen was lower in

summer than in winter (χ^2=4.24, p=0.039, df=1) and it was higher in spring than in winter (χ^2=40.2, p<2.2e-16, df=1,)

Variation between ages

Table 3.8. Variation of haematological parameters between adults and juveniles. The '+' and '–' indicate which age class has the higher or lower values. WBC=White Blood Cells; Lym=Lymphocytes; Het=Heterophils; H/L=Heterophil/Lymphocyte ratio; Eos=Eosinophils; Bas=Basophils; Mon=Monocytes; RBC= Red Blood Cells; Hb= Haemoglobin; PCV=Packed Cell Volume; MCH=Mean Corpuscular Haemoglobin; Fib=Fibrinogen; N.S.=Not Significant; *= p<0.05; ** = p<0.01; *** = p<0.0001.

	WBC	**Lymp**	**Hete**	**H/L**	**Eosi**	**Baso**
Ages	***	N.S.	N.S.	N.S.	*	***
Adults	-	N.S.	N.S.	N.S.	+	+
Juveniles	+	N.S.	N.S.	N.S.	-	-
	Mono	**RBC**	**Hb**	**PCV**	**MCH**	**Fibr**
Ages	**	*	***	**	N.S.	N.S.
Adults	-	+	+	+	N.S.	N.S.
Juveniles	+	-	-	-	N.S.	N.S.

Total leukocyte count was higher in juveniles than in adults (χ^2=12.05, p=0.00050, df=1), in particular in spring (χ^2=5.13, p=0.023, df=1) but not in summer (χ^2=1.69, p=0.193, df=1). Lymphocyte count was not significantly different between juveniles and adults overall (χ^2=2.42, p=0.119, df=1) nor in spring (χ^2=0.05, p=0.81, df=1), but juveniles had higher lymphocyte in summer (χ^2=4.27, n=0.034, df=1).

Heterophil count did not change with age overall (χ^2=0.13, p=0.71, df=1), either in spring (χ^2=3.47, n=0.06, df=1), or summer (χ^2=2.28, p=0.13, df=1). The H/L ratio was not significantly different between juveniles and adults (χ^2=0.17, p=0.678, df=1), either in spring (χ^2=0.20, p=0.65, df=1) or in summer (χ^2=0.53, p=0.46, df=1).

Monocyte count was higher in juveniles than in adults (χ^2=7.95, p=0.0047, df=1), and the trend remained in summer (χ^2=5.55, p=0.018, df=1) but not in spring (χ^2=1.52, p=0.21, df=1). Eosinophil count was overall lower in juveniles than in adults (χ^2=5, p=0.025, df=1), but the difference was not significant when birds were considered only in spring (χ^2=1.54, p=0.21, df=1) or only in summer (χ^2=3.31, p=0.068, df=1). Basophil count was lower in juveniles than in adults (χ^2=30.15, p=3.984e-08, df=1) both in spring (χ^2=13.66, p=0.00021, df=1) and in summer (χ^2=12.49, p= 0.004, df=1).

Juveniles had fewer erythrocytes than adults overall (χ^2=5.93, p= 0.014, df=1) and in spring (χ^2=3.85, p=0.050, df=1), but not in summer (χ^2=0.08, p=0.776, df=1). Haemoglobin was lower in juveniles than in adults (χ^2=48.24, p=3.34e-12, df=1) both in spring (χ^2=9.52, p= 0.0020, df=1) and summer (χ^2=8.08, p=0.0044, df=1). PCV was overall lower in juveniles than in adults (χ^2=9.92, p=0.0016, df=1), and the same trend remained in summer (χ^2=4.27, p=0.04, df=1), but not in spring (χ^2=0.81, p=0.36, df=1).

MCH did not differ between ages overall (χ^2=1.35, p=0.2452, df=1), but in spring juveniles had higher MCH than adults (χ^2=8.59, p=0.0033, df=1), although in summer the difference was no longer significant (χ^2=1.48, p=0.2, df=1). Fibrinogen did not vary overall between adults and juveniles (χ^2=2.41, p=0.11, df=1), but it was higher in juveniles in spring (χ^2=10.80, p=0.001, df=1) but not in summer (χ^2=0.04, t=0.82, df=1).

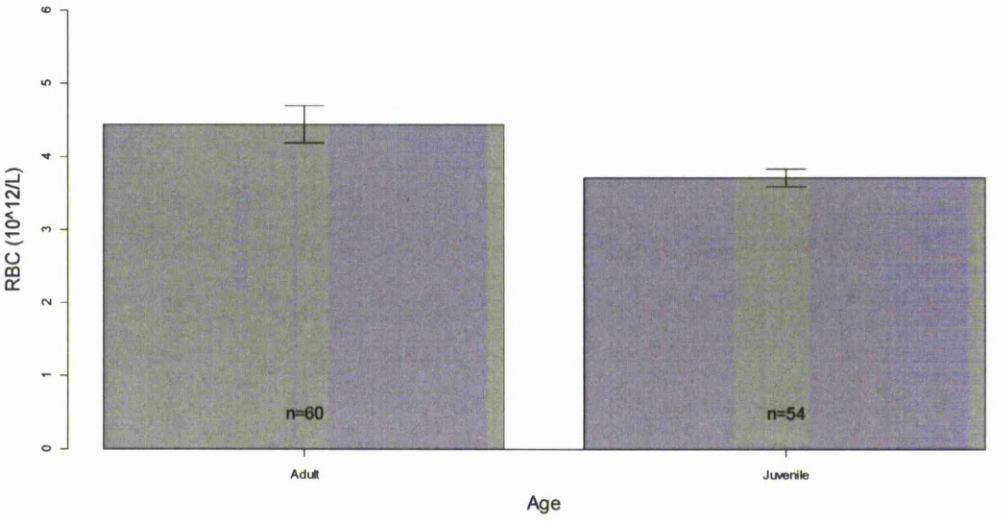

Fig. 3.2. Variation of erythrocytes (RBC) between adults and juveniles. The error bars indicate ± 1 s.e.

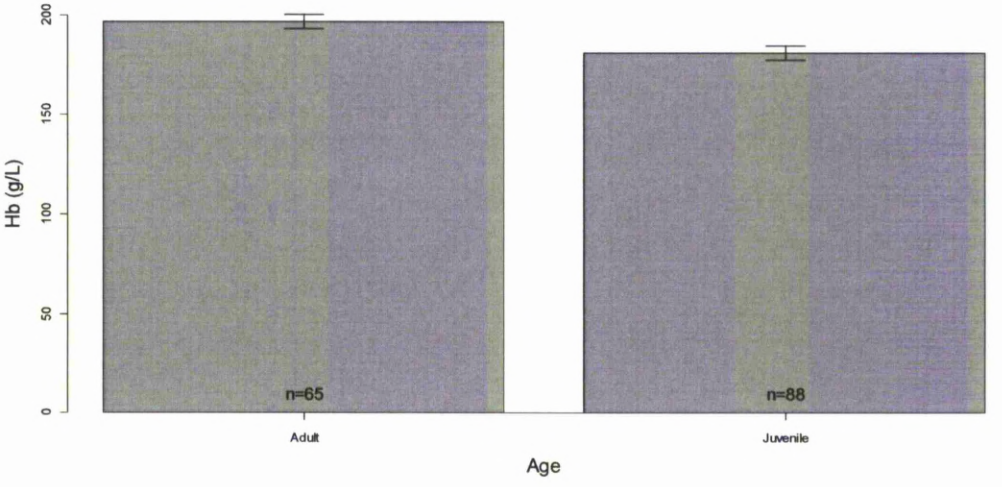

Fig. 3.3. Variation of haemoglobin (Hb) between adults and juveniles. The error bars indicate ± 1 s.e.

Variation between sexes

Table 3.9. Variation of haematological parameters between males and females. The '+' and '–' indicate which sex has the higher or lower values. WBC=White Blood Cells; Lym=Lymphocytes; Het=Heterophils; H/L=Heterophil/Lymphocyte ratio; Eos=Eosinophils; Bas=Basophils; Mon=Monocytes; RBC= Red Blood Cells; Hb= Haemoglobin; PCV=Packed Cell Volume; MCH=Mean Corpuscular Haemoglobin; Fib=Fibrinogen; N.S.=Not Significant; *= p<0.05; ** = p<0.01; *** = p<0.0001.

	WBC	**Lymp**	**Hete**	**H/L**	**Eosi**	**Baso**
Sexes	***	***	***	***	***	***
Male	-	-	-	-	-	+
Female	+	+	+	+	+	-
	Mono	**RBC**	**Hb**	**PCV**	**MCH**	**Fibr**
Sexes	***	***	***	***	***	**
Male	+	-	-	-	+	+
Female	-	+	+	+	-	-

Total WBC count was lower in males than in females (χ^2=129.3, p<2.2e-16, df=1) even when only adult birds were considered (χ^2=33.32, p=7.8e-09, df=1). Males had lower lymphocyte counts than females (χ^2=441.52, p<2.2e-16, df=1), but when only adults were considered males had higher lymphocyte counts than females (χ^2=240.61 , p<2.2e-16, df=1). Heterophil count was significantly lower in males than in females (χ^2=418.75, p<2.2e-16, df=1) even when only adults were considered (χ^2=238.74, p<2.2e-16, df=1). Males had a lower H/L ratio than females (χ^2=320.29, p<2.2e-16, df=1) even when only adults were considered (χ^2=157.08, p<2.2e-16, df=1).

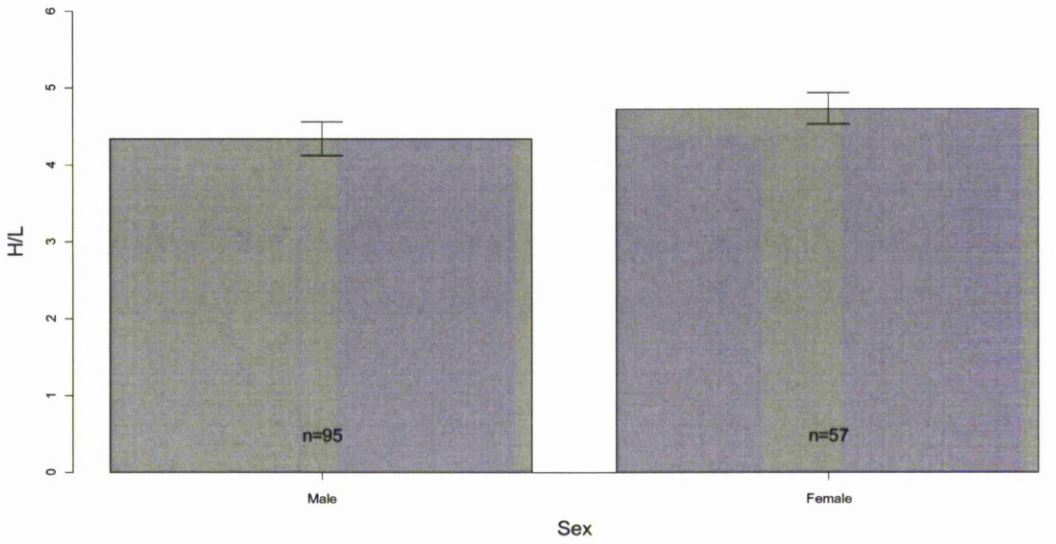

Fig. 3.4. Variation in heterophil to lymphocyte ratio (H/L) between males and females. The error bars indicate ± 1 s.e.

Males had higher monocyte count than females (χ^2=415.01, p<2.2e-16, df=1), even when only adults were considered (χ^2=217.41, p<2.2e-16, df=1). Eosinophil count was lower in males than in females (χ^2=229.02, p<2.2e-16, df=1) even when only adults were considered (χ^2=129.1, p<2.2e-16, df=1). Basophil count was higher in males than females (χ^2=360.74, p<2.2e-16, df=1) but when only adult males were considered basophil count was lower than in adult females (χ^2=180.68, p<2.2e-16, df=1).

Males had lower erythrocyte count than females (χ^2=54.58, p=1.517e-13, df=1) even when only adult birds were considered (χ^2=14.26, p=0.00015, df=1). Haemoglobin was lower in males than females (χ^2=259.79, p<2.2e-16, df=1) even when only adult birds were considered (χ^2=172.44, p<2.2e-16, df=1). Males had lower haematocrit than females (χ^2=26.22, p=3.042e-07, df=1) even when only adults were considered (χ^2=8, p=0.0046, df=1). MCH was higher in males than in females overall (χ^2=115.38, p<2.2e-16, df=1), and the difference remained significant when only adults were considered (χ^2=24.32, p=8.15 e-07, df=1). Fibrinogen was higher in males than in females (χ^2=8.89, p=0.0028, df=1), and the same trend remained when only adults were considered (χ^2=3.92 , p=0.04, df=1).

Variation with condition

Table 3.10. Variation of haematological parameters with condition of the individuals. WBC=White Blood Cells; Lym=Lymphocytes; Het=Heterophils; H/L=Heterophil/Lymphocyte ratio; Eos=Eosinophils; Bas=Basophils; Mon=Monocytes; RBC= Red Blood Cells; Hb= Haemoglobin; PCV=Packed Cell Volume; MCH=Mean Corpuscular Haemoglobin; Fib=Fibrinogen; N.S.=Not Significant; *= p<0.05; ** = p<0.01; *** = p<0.0001.

	WBC	**Lymp**	**Hete**	**H/L**	**Eosi**	**Baso**
Body condition	N.S.	***	***	**	**	***
	Mono	**RBC**	**Hb**	**PCV**	**MCH**	**Fibr**
Body condition	***	N.S.	***	N.S.	N.S.	N.S.

Total WBC count was not significantly correlated with body condition (χ^2=3.22, p=0.07, df=1) in adults (χ^2=0.34, p=0.559, df=1) nor in juveniles (χ^2=1.61, p=0.2, df=1). Lymphocyte count was positively correlated with body condition overall (χ^2=15.93, p=6.562 e-05, df=1) even when only adults were considered (χ^2=8.72, p=0.0031, df=1) or when only juveniles were considered (χ^2=7.72, p=0.0054, df=1). Heterophil count was negatively correlated with condition (χ^2=14.78, p=0.00012, df=1) even when only adults were considered (χ^2=9.26, p=0.0023, df=1) but it was positively correlated with condition in juveniles (χ^2=7.62, p=0.0057, df=1). The lymphocyte/heterophil ratio was negatively correlated with body condition (χ^2=10.57, p=0.0011, df=1) in adults (χ^2=5.32, p=0.021, df=1) and in juveniles (χ^2=5.63, p=0.017, df=1).

Monocyte count was positively correlated with condition (χ^2=17.25, p=3.271e-05, df=1) in both adults (χ^2=9.41, p=0.0021, df=1) and juveniles (χ^2=11.97, p=0.00054, df=1). Eosinophil count was negatively correlated with condition (χ^2=8.61, p=0.0033, df=1) in both adults (χ^2=4.80, p= 0.028, df=1) and in juveniles (χ^2=4.44,

p=0.035, df=1). Basophil count was negatively correlated with overall condition (χ^2=12.94, p=0.00032, df=1) in both adults (χ^2=5.96, p=0.014, df=1) and in juveniles (χ^2=7.59, p=0.0058, df=1).

Total RBC did not vary with the condition of the bird (χ^2=0.86, p=0.35, df=1) even when only adults (χ^2=2.82, p=0.092, df=1) or only juveniles (χ^2=1.36, p=0.24, df=1) were considered. Condition varied across season (χ^2 =16.59, p=0.00085, df=3) and haemoglobin was negatively correlated with condition (χ^2=12.77, p=0.0003, df=1) including when only adults were taken into account (χ^2=12.30, p= 0.00045, df=1) but it was not significant when only juvenile house sparrows were considered (χ^2=1.44, p=0.229, df=1).

Overall MCH did not correlated with condition (χ^2=0.68, p=0.6, df=1), but it was positively correlated with condition when only adults were considered (χ^2=6.49, p=0.01, df=1) but the correlation was not significant when only juveniles were considered (χ^2=2.25, p=0.133, df=1). Condition was not correlated with packed cell volume (χ^2=3.13, p=0.076, df=1) in adults (χ^2=1.96, p=0.157, df=1) nor in juveniles(χ^2=2.13, p=0.14, df=1). Fibrinogen was not correlated with condition (χ^2=0.11, p=0.91, df=1) in adults (χ^2=0.15, p=0.69, df=1) nor in juveniles (χ^2=7e-04, p=0.97, df=1).

Variation with breeding status

There were not enough data to test the relationship between breeding and leukocyte count. Birds that showed evidence of breeding had lower lymphocyte count than birds that were not breeding (χ^2=, p<2.2e-16, df=1). House sparrow showing signs of breeding had higher heterophil count than non-breeding birds (χ^2=, p<2.2e-16, df=1). Breeding birds had higher H/L ratio (χ^2=146.0, p<2.2e-16, df=1), lower lymphocyte count (χ^2=485.3, p<2.2e-16, df=1), higher heterophil count (χ^2=431.5, p<2.2e-16, df=1), higher basophil (χ^2=263.4, p<2.2e-16, df=1) and eosinophil count (χ^2=880.63, p<2.2e-16, df=1) and higher monocytes count (χ^2=, p<2.2e-16, df=1) than non breeding birds.

Table 3.11. Variation of haematological parameters between breeding and non-breeding birds. The '+' and '–' indicate which breeding status has the higher or lower values. WBC=White Blood Cells; Lym=Lymphocytes; Het=Heterophils; H/L=Heterophil/Lymphocyte ratio; Eos=Eosinophils; Bas=Basophils; Mon=Monocytes; RBC= Red Blood Cells; Hb= Haemoglobin; PCV=Packed Cell Volume; MCH=Mean Corpuscular Haemoglobin; Fib=Fibrinogen; NED=Not Enough Data; *= $p<0.05$; ** = $p<0.01$; *** = $p<0.0001$.

	WBC	**Lymp**	**Hete**	**H/L**	**Eosi**	**Baso**
Breeding	NED	***	***	***	***	***
Breeding	NED	-	+	+	+	+
Non-breeding	NED	+	-	-	-	-
	Mono	**RBC**	**Hb**	**PCV**	**MCH**	**Fibr**
Breeding	***	NED	***	NED	NED	NED
Breeding	+	NED	+	NED	NED	NED
Non-breeding	-	NED	-	NED	NED	NED

There were not enough data to investigate the relationship between erythrocyte count and breeding status. Breeding birds had higher haemoglobin (χ^2=883.82, p<2.2e-16, df=1) than non-breeding birds. There were not enough data to investigate the relationship between haematocrit and breeding status. There were not enough data to test a difference in MCH between breeding and non-breeding individuals. There were not enough data to test a difference in fibrinogen between breeding and non-breeding individuals.

Variation with moult status

Moulting birds had higher leukocyte count than non-moulting birds (χ^2=378.99, p<2.2e-16, df=1), a trend that was consistent in adults (χ^2=113.53, p<2.2e-16, df=1) and juveniles (χ^2=267.73, p<2.2e-16, df=1). Moulting birds had higher lymphocyte count than non-moulting birds (χ^2=636.82, p<2.2e-16, df=1), which was consistent when only adults were considered (χ^2=223.63, p<2.2e-16, df=1) but the trend was reversed when only juveniles were considered (χ^2=414.02 p<2.2e-16, df=1).

Moulting birds had lower heterophil count than non-moulting birds (χ^2=621.59, p<2.2e-16, df=1), which was true also when only adults were considered (χ^2=221.77, p<2.2e-16, df=1) but juveniles showed the opposite trend, with moulting birds having a higher heterophil count than non-moulting ones (χ^2=400, p<2.2e-16, df=1). Moulting birds had a higher H/L ratio than non-moulting birds (χ^2=492.33, p<2.2e-16, df=1), which was consistent in adults (χ^2=200.59, p<2.2e-16, df=1) but not in juveniles, in which moulting birds had lower H/L ratio than non-moulting juveniles (χ^2=301.05, p<2.2e-16, df=1).

Table 3.12. Variation of haematological parameters between moulting and non-moulting birds. The '+' and '–' indicate which moult status has the higher or lower values. WBC=White Blood Cells; Lym=Lymphocytes; Het=Heterophils; H/L=Heterophil/Lymphocyte ratio; Eos=Eosinophils; Bas=Basophils; Mon=Monocytes; RBC= Red Blood Cells; Hb= Haemoglobin; PCV=Packed Cell Volume; MCH=Mean Corpuscular Haemoglobin; Fib=Fibrinogen; NED=Not Enough Data; *= p<0.05; ** = p<0.01; *** = p<0.0001.

	WBC	**Lymp**	**Hete**	**H/L**	**Eosi**	**Baso**
Moult	***	***	***	***	***	***
Moulting	+	+	-	+	+	-
Non-moulting	-	-	+	-	-	+
	Mono	**RBC**	**Hb**	**PCV**	**MCH**	**Fibr**
Moult	***	***	***	***	***	***
Moulting	-	+	+	-	+	-
Non-moulting	+	-	-	+	-	+

Moulting birds had lower monocyte count than non-moulting ones (χ^2=554.85, p<2.2e-16, df=1), and this relationship was true when only adults were considered (χ^2=199.14, p<2.2e-16, df=1) but it was the opposite for juveniles in which moulting birds had higher monocyte count than non-moulting birds (χ^2=354.35, p<2.2e-16, df=1). Moulting birds overall had higher eosinophils than non-moulting ones (χ^2=433.25, p<2.2e-16, df=1), even when only juveniles were considered (χ^2=226.92, p<2.2e-16, df=1), but moulting adults had lower eosinophil count than non-moulting

ones (χ^2=236.24, p<2.2e-16, df=1). Moulting birds overall had lower basophil count than non-moulting birds (χ^2=616.02, p<2.2e-16, df=1), both when only adults (χ^2=223.63, p<2.2e-16, df=1) and only juveniles (χ^2=399.4, p<2.2e-16, df=1) were considered.

Moulting birds had higher total erythrocyte count (χ^2=276.83, p<2.2e-16, df=1). Adult birds that were moulting had a lower erythrocyte count (χ^2=36.63, p=1.425e-09, df=1) than non-moulting adults, but juveniles that were moulting had a higher erythrocyte count (χ^2=212.09, p<2.2e-16, df=1) than non-moulting juveniles. Birds undergoing moult had higher haemoglobin level (χ^2=330.01, p<2.2e-16, df=1) than those not moulting. The same trend occurred when adult birds were considered (χ^2=143.71, p<2.2e-16, df=1) but it was opposite for juvenile birds, for which individuals moulting had lower haemoglobin than those not-moulting (χ^2=226.92, p<2.2e-16, df=1). Packed cell volume was lower in moulting juveniles (χ^2=185.25, p<2.2e-16, df=1) than in non-moulting birds but there were not enough data to investigate difference in PCV between moulting and unmoulting adults. Moulting birds overall had higher MCH than non-moulting birds (χ^2=556.61, p<2.2e-16, df=1), a trend that remained significant when only adults were considered (χ^2=182.09, p<2.2e-16, df=1), although moulting juveniles had lower MCH than non-moulting juveniles (χ^2=379.11, p<2.2e-16, df=1). Moulting birds had more fibrinogen than non-moulting individuals (χ^2=125.94, p<2.2e-16, df=1), even when only juveniles (χ^2=89.95, p<2.2e-16, df=1) but moulting adults had lower fibrinogen than non-moulting adults (χ^2=56.03, p=7.121e-14, df=1).

3.4.3 Bacteriology

Bird tables and bird feeders of 23 non-intensive sites, and 12 intensive sites were swabbed fortnightly and weekly, respectively, for a total of 406 swabs. None of the swabs tested positive for *Salmonella* or *Escherichia coli* bacteria, 2 swabs belonging to two different sites one in February and one in May tested positive for *Yersinia enterocolitica* , and a third swab coming from a third site tested positive for *Pseudomonas aeruginosa* in December.

Only one house sparrow out of the 271 tested was positive for *Salmonella* Thyphimurium DT56 (Laboratory of Enteric Pathogens, Health Protection Agency). The bird was sampled in May and came from a different site from the ones tested positive for *Y. enterocolitica* and *P. aeruginosa.*

3.4.4 Haemoparasites

The total prevalences of *Plasmodium* spp. (Fig. 3.5)., microfilaria and *Leucocytozoon* spp., and prevalence among adults and juveniles are reported in table 3.6.

Table 3.13. Prevalence of *Plasmodium* spp.., *Leucocytozoon* spp., and microfilaria in all sites, in adults from all sites only, and in juveniles from all sites only expressed as percentage and as proportion (in brackets).

Haemoparasite	**Total prevalence**	**Adult prevalence**	**Juvenile prevalence**
Plasmodium spp.	21.8% (83/380)	29.7% (55/185)	14.4% (28/195)
Leucocytozoon spp.	<1%(1/380)	<1% (1/185)	0
Microfilaria	<1% (1/380)	0	<1% (1/195)

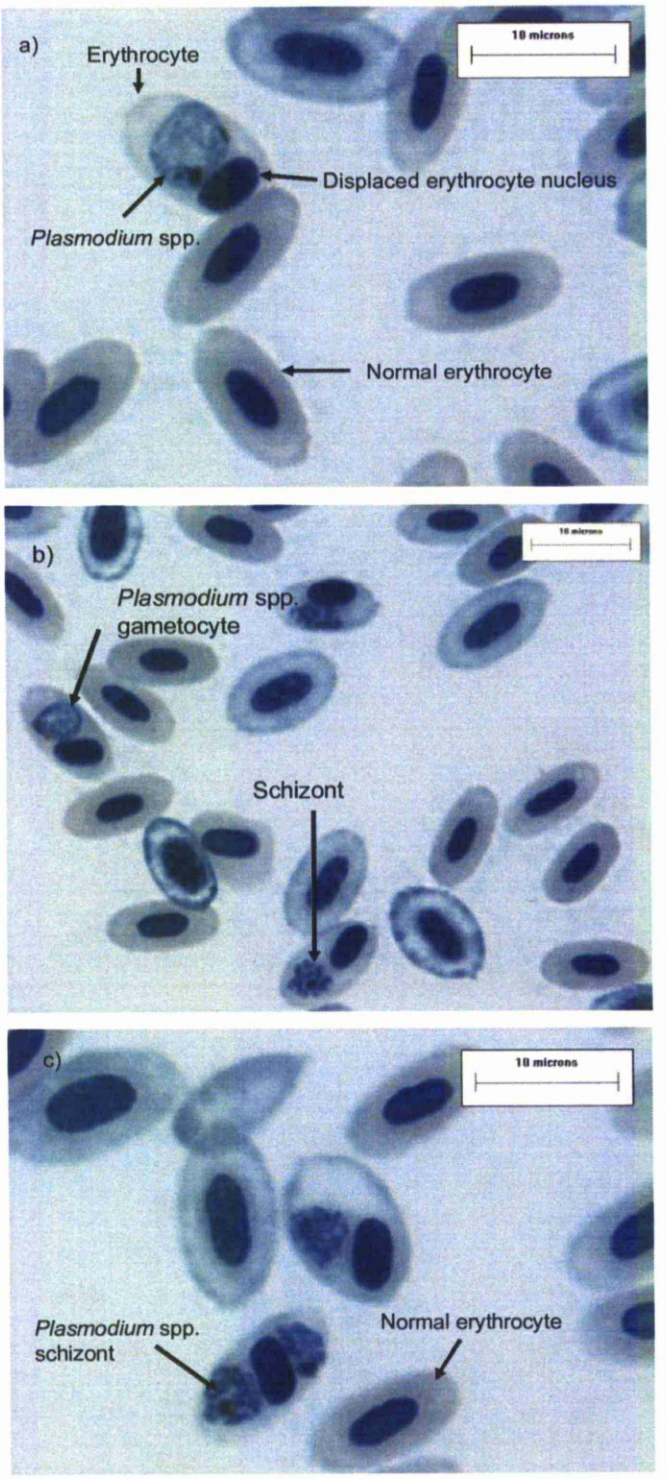

Fig. 3.5. *Plasmodium* spp. (a, b and c). *Plasmodium* spp. can be recognised by the nucleus displacement of the infected erythrocyte, and the presence of the asexually-reproducing phase of the parasite called schizonts, which is diagnostic for infection with this parasite (Atkinson, 2008). (Photos: Daria Dadam).

Plasmodium spp.

The prevalence of species of *Plasmodium* did not vary significantly between sites (χ^2 =18.07, p= 0.064, df=10), but it was lower in juveniles than in adults (χ^2=12.02, p=0.0006, df=1). The intensity of *Plasmodium* spp infection was significantly different across sites (χ^2 =1703, df=9, p=2.94-7) .

Individuals infected with *Plasmodium* had lower total leucocyte counts than non-infected birds (χ^2=39.56, p =3.17e-10, df=1), and leucocyte count decreased with intensity of *Plasmodium* infection (χ^2=7.38, p=0.006, df=1). Birds infected with *Plasmodium* had higher H/L ratio than non-infected birds (χ^2=11.14, p=0.008, df=1), but intensity of infection was negatively correlated with H/L ratio (χ^2=23.24, p=1.027e-06, df=1).

Monocytes percentage decreased in infected birds compared to non-infected ones (χ^2=17.13, p=3.48e-5, df=1), but increased with intensity of infection (χ^2=14.98, p=0.0001, df=1). Lymphocyte percentage was higher in infected birds than in non-infected ones (χ^2=20.21, p= 6.93e-09, df=1) but it decreased with intensity of the infection (χ^2=28.59, p= 8.93e-08, df=1). Heterophil percentage was lower in infected birds than in non-infected birds (χ^2=17.13, p= 3.48e-05, df=1) but increased with intensity of infection (χ^2=24.02, p= 9.52e-08, df=1). Basophil percentage was higher in infected birds (χ^2=19.20, p=1.174e-5, df=1) but it decreased with intensity of infection (χ^2=19.74, p=8.85e-06, df=1). Eosinophil percentage was higher in infected birds (χ^2=13.54 , p=0.0023 df=1) and it also increased with increasing infection (χ^2=37.88 , p=7.518e-10 , df=1). Fibrinogen was lower in *Plasmodium*-infected birds (χ^2=3.85, p= 0.049, df=1) and it decreased with *Plasmodium* intensity (χ^2=7.82, p=0.0051, df=1).

Total erythrocyte count was positively associated with *Plasmodium* prevalence (χ^2=5.48, p= 0.019, df=1) and with intensity of infection (χ^2=6.04, p= 0.013, df=1). Haemoglobin quantity was negatively correlated with *Plasmodium* prevalence (χ^2=11.57, p=0.0006, df=1) and with intensity of the infection (χ^2=9.73, p=0.0018, df=1). The mean corpuscular haemoglobin (MCH) was negatively associated with *Plasmodium* prevalence (χ^2=23.99, p=9.67 e-07, df=1) and intensity of infection (χ^2=48.30, p=3.634e-12, df=1). Similar pattern was found for the mean corpuscular

haemoglobin concentration (MCHC) which was negatively associated with *Plasmodium* prevalence (χ^2= 11.46, p= 0.00071, df=1) and it decreased with *Plasmodium* intensity (χ^2=30.51 , p=3.319e-08, df=1). The packed cell volume (or haematocrit) (PCV or Hct) was higher in birds with *Plasmodium* (χ^2=6.79, p= 0.009, df=1) and it increased with intensity of the infection (χ^2=19.42, p= 1.049e-05, df=1). The mean corpuscular value (MCV) was also lower in the presence of *Plasmodium* (χ^2=8.22, p=0.004, df=1) but it increased with *Plasmodium* infection level (χ^2=26.78 p= 2.28e-07, df=1).

3.4.5 Gastro-intestinal parasites

The total prevalence of *Isospora* spp., *Atoxoplasma* spp. and helminths parasites (Fig 3.6) are shown in Table 3.7. Co-infection with *Isospora* spp. and *Plasmodium* spp. occurred in 3/242 birds, and *Plasmodium* spp. and *Atoxoplasma* spp. in 26/376 birds. Despite their taxonomic identity (Barta *et al.*, 2005), *Isospora* spp. and *Atoxoplasma* spp. co-infection occurred in only 1/242 bird, while 13/242 showed infection with *Isospora* spp. but not *Atoxoplasma* spp., 62/242 showed infection with *Atoxoplasma* spp. but not *Isospora* spp., and 115/262 birds showed no infection from either form of the parasite.

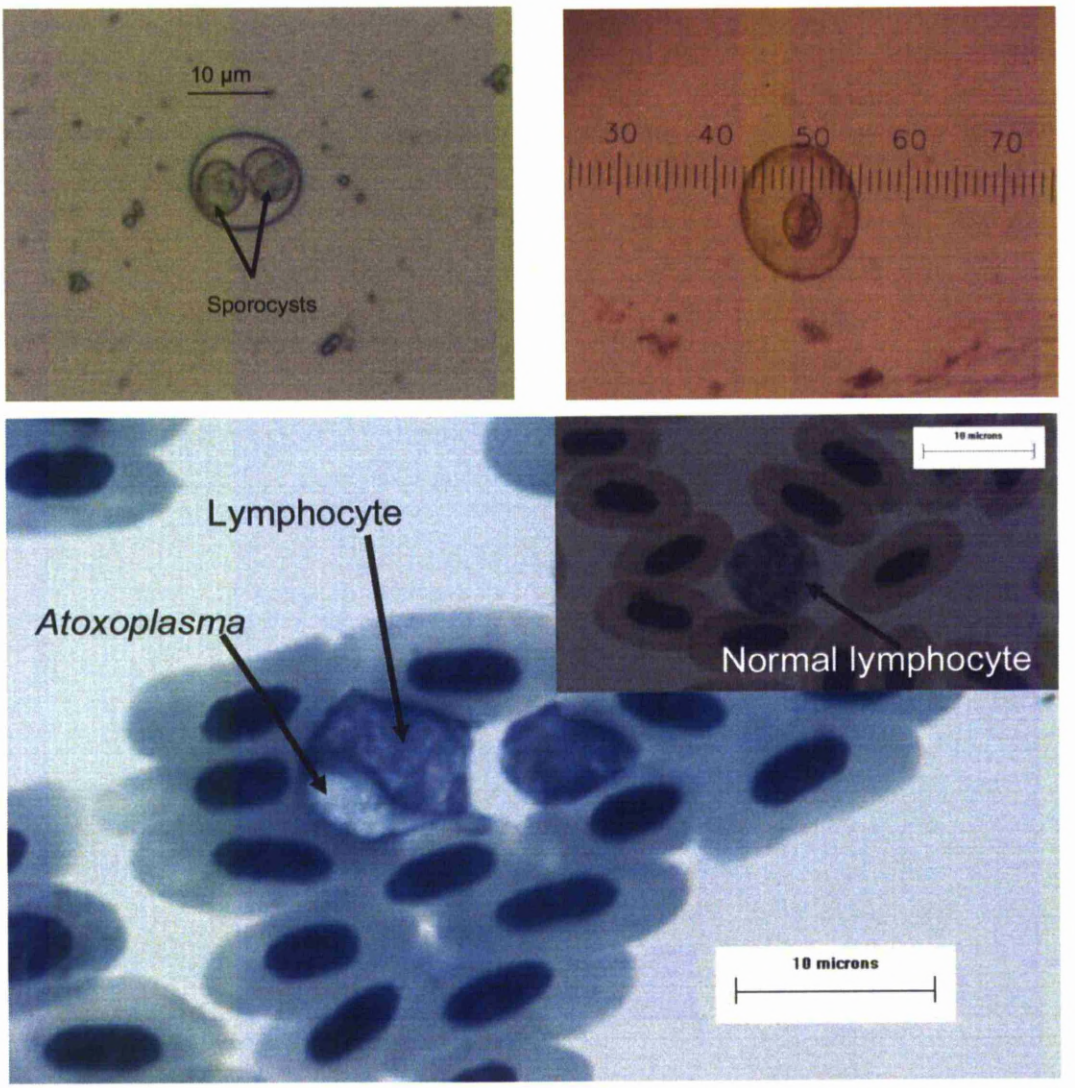

Fig. 3.6. *Isospora* spp. (top left), helminth parasite egg (top right) (10x), and *Atoxoplasma* spp. (bottom). *Isospora* spp. and *Atoxoplasma* spp. are thought to be different stages of the same coccidian parasite (Barta *et al.*, 2005), which is identified as *Isospora* when found in the gastro-intestinal tract, but *Atoxoplasma* when found in the blood, where it parasitise leucocytes (Campbell and Ellis, 2007). The two sporocysts identify the coccidia oocyst as belonging to the genus *Isospora*, while the hooks inside the helminth egg identify it as a tapeworm but the species was not ascertained. (Photos: Daria Dadam).

Table 3.14. Prevalence of *Isospora* spp. , *Atoxoplamsa* spp., and tapeworm eggs in all sites, in adults from all sites only, and in juveniles from all sites only expressed as percentage and as proportion (in brackets).

Parasite	Total prevalence	Adult prevalence	Juvenile prevalence
Isospora spp.	31.4% (76/242)	27.2% (28/103)	34.5% (48/139)
Atoxoplasma spp.	30% (113/376)	28% (51/182)	31.2% (62/194)
Tapeworm eggs	3.7% (9/242)	7.7 % (8/103)	0.7 % (1/139)

Isospora spp

The prevalence of *Isospora* spp. was significantly different between sites (χ^2 = 24.74, p=0.0054, df=10), but it was not significantly different between adults and juveniles (χ^2 =1.24, p=0.27, df=1). The intensity of *Isospora* infection was not different across sites (χ^2 =1.22, p=0.74, d.f.=3).

The total leucocyte count was higher in birds that were shedding *Isospora* spp oocysts than in birds that did not (χ^2 =432.03, p< 2.2e-16, df=1) but the sample size was not enough to check for any differences in intensity. Birds shedding *Isospora* spp oocysts had lower lymphocyte count (χ^2 =844.31 p<2.2e-16, df=1), but higher heterophil count (χ^2 =812.14, p<2.2e-16, df=1), and consequential higher H/L ratio (χ^2 =604.85, p< 2.2e-16, df=1) than birds that did not shed *Isospora* spp. oocysts. Monocyte count was higher in birds shedding *Isospora* spp oocysts (χ^2 =745.59, p< 2.2e-16, df=1). Basophil count (t=750.46, p<2.2e-16, df=1) and eosinophil count (χ^2 = 585.23, p<2.2e-16, df=1) were significantly lower in birds shedding Isospora spp oocysts.

Total erythrocyte count was higher in birds shedding isospora spp oocysts (χ^2 =313.34, p= 2.2e-16, df=1) , as was haemoglobin quantity (χ^2 =464.68, p<2.2e-16, df=1), and PCV (χ^2 =248.84, p<2.2e-16, df=1) The mean corpuscular volume (MCV) was lower in birds shedding Isospora spp oocysts (χ^2 =295.65, p<2.2e-16, df=1), as was the mean corpuscular haemoglobin (MCH) (χ^2 =536.03, p< 2.2e-16, df=1), and the mean corpuscular haemoglobin concentration (MCHC) (χ^2 =387.33, p<2.2e-16, df=1).

Fibrinogen quantity was higher in birds shedding Isospora spp oocysts (χ^2 =124.64, p<2.2e-16, df=1).

Atoxoplasma spp.

The prevalence of *Atoxoplasma* spp. varied across sites (χ^2=34.11, p=0.0001, df=10) and intensity of the parasite also differed across sites (χ^2=33.14, p=0.00012, df.=9). The prevalence of the parasite did not differ between ages (χ^2=1.55, p=0.20, df=1).

Total leucocyte count was higher in house sparrows infected with *Atoxoplasma* than in non-infected birds (χ^2=25.54, p= 4.316e-07, df=1) and it increased with intensity of infection (χ^2=18.70 , p=1.527e-05, df=1). The H/L ratio was lower in birds that were positive for *Atoxoplasma* spp infection than in non-infected birds (χ^2=10.96, p=0.0009, df=1) but it increased with the intensity of the infection (χ^2=14.19, p=0.00016, df=1). Lymphocyte percentage was higher in parasitised individuals than in non-parasitised birds (χ^2=16.65, p=4.478e-05, df=1) but it decreased with intensity of the infection (χ^2=339.50, p<2.2e-16, df=1). Heterophil percentage was higher in parasitised birds than in non-parasitised birds (χ^2=14.28, p= 0.00015, df=1) and it increased with intensity of infection (χ^2= 22.91, p=1.69e-06, df=1).

Monocyte percentage was lower in birds exhibiting circulating *Atoxoplasma* spp (χ^2=14.25 p= 0.00015, df=1) and decreased with intensity of infection (χ^2=21.80, p=3.02e-06 , df=1). Basophil percentage was lower in birds with *Atoxoplasma* spp (χ^2=13.88, p=0.00019, df=1) but it increased with intensity of infection (χ^2=24.78 , p=6.36e-07, df=1). Eosinophil percentage was higher in individuals with *Atoxoplasma* spp (χ^2=14.74, p=0.00012, df=1) and it increased with intensity of infection (χ^2=20.04, p=7.557e-06, df=1). Fibrinogen quantity was higher in birds that were infected with *Atoxoplasma* spp than in non-infected birds (χ^2=5.37, p=0.02, df=1) but did not vary with intensity of the infection (χ^2= 3.26, p=0.07 , df=1).

Total erythrocyte count was lower in birds with *Atoxoplasma* spp (χ^2=10.55, p= 0.0011, df=1) but it did not vary with intensity of infection (χ^2=3.78, p=0.051, df=1). Haemoglobin quantity was lower in birds with *Atoxoplasma* spp infection (χ^2=44.22, p= 2.93e-11, df=1) and it was negatively correlated with the increase intensity of infection (χ^2=54.13, p=1.86e-13, df=1). Mean corpuscular haemoglobin (MCH) was

higher in birds with *Atoxoplasma* spp (χ^2=25.85, p=3.68e-07, df=1) but it decreased with intensity of infection (χ^2=24.18, p=8.75e-07, df=1). The mean corpuscular haemoglobin concentration (MCHC) was lower in individuals with *Atoxoplasma* spp (χ^2=12.52, p= 0.0004, df=1) and it increased with intensity of infection (χ^2=10.19, p= 0.0014, df=1). The packed cell volume (or haematocrit) (PCV or Hct) was higher in individuals with *Atoxoplasma* spp infection (χ^2=5.94, p=0.014, df=1) and it increased with intensity of infection (χ^2=5.38 , p=0.02, df=1). The mean corpuscular volume (MCV) was higher in birds with *Atoxoplasma* spp (χ^2=16.58, p= 4.66e-05, df=1) and it increased with intensity of infection (χ^2=16.22, p=5.63e-05, df=1).

Tapeworm eggs

The prevalence of tapeworm eggs was lower in juveniles than in adults (χ^2 =8.84, p=0.002, df=1) but the sample size was too small to test differences at the site level or interactions with haematological values.

3.5 DISCUSSION

3.5.1 Basal haematological values

The total mean leukocyte count was 13.11 x10^9 /L , which was higher that the suggested normal range of 4-10x10^9 /L (Campbell and Ellis, 2007; Samour, 2006) but the reason for this discrepancy is not clear. The highest value in this study was almost 5 times as high as the maximum value considered normal in most species, indicating that there were some birds with very high counts, although they had not tested positive for any of the parasites considered, nor any bacteria. A previous study (Puerta *et al*, 1995) had found the highest value for leucocytes to be 29.5 x10^9 /L, although the authors had not checked for presence of infection that could have increased the count.

The lymphocyte mean percentage of 49.92 was within the 30-60% normal range (Campbell and Ellis, 2007; Samour, 2006), although the highest percentage found in this study (90%) was higher than the top range of the normal values, but it was similar to the maximum of 81.3% found in rufus-collared sparrows (*Zonotrichia capensis*) (Ruiz *et al.*, 2002). The mean number of lymphocytes was 6.78x10^9 /L ,

which was higher than the range of 1-4x10^9 /L suggested in the literature for similar species (Campbell and Ellis, 2007; Samour, 2006).

The total heterophil mean percentage was 21.37 and within the ranges of 20-60% (Campbell and Ellis, 2007; Samour, 2006; Ruiz *et al.*, 2002), although the lowest and the highest values (2% and 74%, respectively) were outside this range. The mean number of heterophils was 2.69x10^9 /L and within the range of 0.1-4.9x10^9 /L indicated in the literature (Campbell and Ellis, 2007; Samour, 2006).

The average monocyte percentage was 6.9%, which was higher than the 0-1% indicated in previous studies (Campbell and Ellis, 2007; Samour, 2006), and the total number of monocytes was 2.04x10^9 /L , which was also higher than the 0-0.3 x10^9 /L indicated in the literature (Campbell and Ellis, 2007; Samour, 2006). However, previous studies have found a mean monocyte percentage of 3%, and a maximum of 5.7% (Ruiz *et al.*, 2002), indicating that the 1% suggested by some references (Campbell and Ellis, 2007; Samour, 2006) may be too much of a generalisation.

Eosinophil and basophil mean percentages were 2.16% and 9.6%, respectively, indicating that both values were also higher than the range of 0-1% and 0-5%, respectively, presented in the literature (Campbell and Ellis, 2007; Samour, 2006). However, other authors have also found percentage of eosinophils to be higher than the general normal range, with a mean of 2.3% and a maximum of 3.7%, and for basophils a mean of 6.3% and a maximum of 9.4% (Ruiz *et al.*, 2002). The mean number of eosinophils and basophils in this study were 0.3x10^9 /L and 1.29x10^9 /L , respectively, which were higher than those suggested as normal ranges of 0-0.03x10^9 /L and 0-0.04x10^9 /L (Campbell and Ellis, 2007; Samour, 2006). However, the lower range of the number of circulating basophils and eosinophils found in this study are consistent with those found by previous authors (Campbell and Ellis, 2007; Samour, 2006).

The mean total value of circulating erythrocytes was 4.09x10^9 /L, which is consistent with the ranges found in the literature (Campbell and Ellis, 2007; Samour, 2006; Puerta *et al.*, 1995). The mean PCV was 48.9%, which is also within the normal ranges of 35-55% (Campbell, 1995) and it did not suggest anaemia; however, the

lowest value found in this study of 27% is suggestive of anaemia (Campbell, 1995), as is the lowest value of Hb of 107g/L [values below 111g/L suggest anaemia (Samour, 2006)], but these value came from two different individuals. The mean total value for Hb was higher than the normal range reported in the literature, and the highest value of 254 g/L is the highest value for Hb reported in a clinically healthy wild bird, to the author's knowledge.

The mean total value for fibrinogen was 2.42g/L and within the published normal ranges of 0.9-4 g/L (Campbell and Ellis, 2007; Samour, 2006), although the highest value of 14.29 g is very high for a clinically healthy bird, indicating that there either was an undetected infection in the individual, or that the technique used yielded an erroneous result in that instance, a possibility considering that the bird was one of the first ones for which fibrinogen was used, and it is plausible that an initial measuring mistake might have occurred.

Overall, the basal haematological values suggest higher mean values and range of total leucocytes, in particular monocytes, basophils and eosinophils, than previously suggested for similar size birds (Campbell and Ellis, 2007; Samour, 2006). The mean percentage of lymphocytes was higher than the mean percentage of heterophils, which is in accordance with other studies (Davis *et al.*, 2008; Sturkie and Giminger, 1986).

Haemoglobin values were also higher than those reported in the literature. A possible explanation for this may be a higher level of hypoxia than in previous studies, possibly connected to CO_2 pollution in a large city such as London compared to rural areas where previous studies had been conducted. Alternatively, higher Hb levels may be due to general dehydration of the birds, although the haematocrit values were generally within the normal range, so an hypothesis pointing at dehydration does not seem to have support.

3.5.2 Haematological changes between years, season, ages, sexes, breeding, moulting, and condition of non-parasitised individuals

Variation between years

Haematological values generally did not change between years. Among the leucocyte-related parameters, only the H/L ratio, the heterophil count, the eosinophil count, and basophil count changed between years, but these parameters are known to change according to stress levels (e.g Maxwell, 1993) and they are likely to have low between-years repeatability across a pooled sample of all birds from all sites. Leukocytes are also reported to respond to multiple external transient environmental stimuli (Campbell, 1995; Campbell and Ellis, 2007) which can change from year to year. It is therefore expected that leukocyte types vary across years, and that values associated to erythrocytes remain more constant. However, Norte and colleagues (Norte *et al.*, 2008) found that leucocyte-related parameters had high repeatability even between years, but their study was based on repeated sample of individuals from only one site. Kilgas and colleagues (Kilgas *et al.*, 2006) found differences in H/L ratio between years. Total erythrocyte count, haemoglobin quantity, MCH and packed cell volume did not vary across years, suggesting that the factors affecting these values had remained constant across the three years of the study, although between years variation of haematocrit was found in great tits (*Parus major*) (Kilgas *et al.*, 2006).

Variation between seasons

Haematological values generally varied across seasons. Total leukocyte count was higher in summer than in spring, but none of the other season by season differences were significant. Lymphocytes were higher in summer than in autumn and than in winter, possibly due to an age-confounding factor associated with a high number of juveniles in summer, which had higher lymphocyte counts than adults, but only in summer, although this result has to be interpreted with caution due to the discrepancy in sample size between adults and juveniles in summer. Heterophil count varied across seasons overall, but the only significant difference between season dyads was a lower count in winter than in autumn. The heterophil/lymphocyte ratio (H/L) differed across seasons overall, but the only significant difference was a higher H/L

ratio in summer than in winter, contrary to a study on great tits that found that H/L ratio was higher in spring (Norte *et al.*, 2009).

Monocyte count did not vary across seasons overall, but it was higher in winter than in summer and in autumn than in winter, contrary to basophils that were higher in winter than in autumn, and across seasons overall. Eosinophils did not vary overall, but their count seemed to oscillate between seasons, since it was the highest in summer, then decreased in autumn, to decrease again in winter (but remaining lower than in summer) but did not significantly change between winter and spring or spring and summer. This may suggest that the increase was so slow across winter and spring that only the difference between summer and winter, but not winter and spring or spring and summer was significant at a statistical level. Spring is a transition season encompassing the beginning of the breeding season (March), when haematological changes may not yet be apparent, and the period when the breeding effort is fully under way (May-June).

Total RBC did not change across seasons overall, but it was higher in spring than summer, and PCV was the highest in spring and lowest in summer. PCV increased between winter and spring, then dropped in summer, to increase again in autumn, and once again in winter. There is, therefore, a stable increase in PCV value from the lowest point in summer to the highest in spring. These results are in accordance with a previous study on great tits that found that haematocrit was the highest in spring (Norte *et al.* 2009), and with other studies that have found haematocrit values higher in winter than in summer (Kostelecka-Myrcha, 1997; Ruiz *et al.*, 1995; Swanson, 1991; Clemens, 1990; Swanson, 1990; Rehder and Bird, 1983; deGraw *et al.*, 1979; Carey and Morton, 1976), although a study on Australian passerines found no seasonal variation in PCV and Hb (Breuer *et al.*, 1995).

An explanation suggested for the higher haematocrit in winter is in the need for thermoregulation (Carey and Morton, 1976), and to meet the higher energy demands of the cold season (Rosemann and Ruiz, 1993). An alternative explanation may be that juveniles act as confounding factor in the summer estimates. In summer the high number of juveniles, which in this study had lower total erythrocyte count and lower PCV, in accordance with previous studies (Simmons and Lill, 2006; Shapiro *et al.*,

1999; Kostelecka-Myrcha *et* al., 1997; Merino and Barbosa, 1997; Puerta *et al.*, 1995), may artificially lower the total value of erythrocyte and haematocrit. An important aspect to consider is that haematocrit and total erythrocyte count may not be accurate estimates of oxygen transportation capacity, since both values can be unaffected by the presence of immature erythrocytes (reticulocytes) which can be produced quickly through erythropoiesis (Campbell, 1995; Dein, 1986) but that contain about 20% of the haemoglobin of mature erythrocytes (Jain, 1993). In this study haemoglobin concentration was the highest in winter, and declined throughout the season to reach its lowest level in autumn.

Several studies have highlighted a possible link between increased workload and higher oxygen demand to the tissues, for example during physical demanding seasons such as the breeding period (Davey *et* al., 2000; Hõrak *et al.*, 1998; Saino *et al.* 1997; Clemens, 1990; Jaeger and McGrath, 1974). In this study haemoglobin and MCH were equally high in winter and spring, and in winter and summer, contrary to a previous study on great tits that found that Hb was low in spring and high in winter (Norte *et al.*, 2009). Based only on seasonal variation found in this study, the hypothesis linking Hb to physical demand is not supported. However, if the need for oxygen was higher during the breeding season in house sparrows due to the higher demands of breeding, one would expect breeding birds to have higher Hb than non-breeding birds. This hypothesis is supported by the results in this study, in which breeding birds had higher Hb levels than non-breeding adults.

Quantity of fibrinogen in the current study showed higher levels in spring than in winter, and higher in winter than in summer. These results are unexpected because other authors had suggested that fibrinogen did not show seasonal variation (Samour and Howlett, 2008; Jain, 1993). It is possible that fibrinogen variation between seasons is due to a high variability at an individual level, exacerbated by the relatively small sample size when single seasons are considered.

Variation between ages

Juveniles had higher overall leukocyte count than adults, in accordance with previous studies (Davis *et al.*, 2004; Nava *et al.*, 2001; Puerta *et al.*, 1995). Specifically, juveniles had higher lymphocyte and monocyte counts than adults but only in

summer. These results are in accordance with other studies (Davis *et al.*, 2004; Nava *et al.*, 2001; Fairbrother and O'Loughling, 1990), and they may be due to their naive immune system experiencing a higher number of novel antigens than adults (Puerta *et al.*, 1995). Juveniles had lower eosinophils and basophils than adults, but there was no difference in heterophil counts or H/L ratio between the two age classes. These results do no agree with previous studies which had found that juveniles had higher basophil (Nava *et al.*, 2001) and higher heterophil counts (Figuerola *et al.*, 1999; Alonso *et* al., 1991; Hawkey *et* al., 1983; but see Fairbrother and O'Loughing, 1990) but lower H/L ratio (Norte *et al.*, 2009) than adults.

Lower eosinophils in juveniles than in adults may suggest a higher level of stress in juveniles since a lower level of this type of leukocyte has been linked to stress (Jein, 1986). This result is in contrast with a study on cirl buntings (*Emberiza cirlus*) that found higher eosinophils in adults (Figuerola *et al.*, 1999). One aspect to consider is, however, the inability to distinguish juveniles from adults after the end of the postjuvenile and postnuptial moult (Svensson, 1992, see section 2.2.1). From October onwards the two age classes are not distinguishable, but the haematological values will differ for two additional months (Samour, 2000) so the lack of difference between the two age classes may be due to the lower heterophil count of some juveniles being accounted in the adult age class after the complete postjuvenile moult.

Juveniles had lower RBC than adults overall, but only in spring, while in summer the difference was no-longer significant. PCV was also overall lower in juveniles than adults. The lack of difference of RBC and PCV between sexes in summer may be due to the very young age of juveniles in spring (April-June), while among the juveniles in summer (July-September) there are birds that are already several weeks old, especially towards the end of the season. Some authors have found that RBC, PCV, and Hb increased with age (Shapiro *et al.*, 1999; Kostelecka-Myrcha, 1997; Kostelecka-Myrcha *et al.*, 1997; Puerta *et al.*, 1995), but Ots and colleagues (Ots *et al.* 1998) did not find a correlation between haematocrit and age. Juveniles had lower Hb values than adults both in spring and in summer, in accordance to a previous study on great tits (Norte *et al.*, 2009). These results partially reflect the lower number of erythrocytes, and they may also be due to the high level of Hb in adults

during summer, potentially linked to increased oxygen demand of the breeding season. MCH did not vary between ages, in accordance with a previous study that found constant MCH throughout the development of nestling of hirundines (Simmons and LIll, 2006).

Fibrinogen did not vary overall between adults and juveniles, but it was higher in juveniles in spring but not in summer. This result is partially unexpected because other authors had suggested that fibrinogen did not vary with age (Samour and Howlett, 2008; Jain, 1993); however, the difference may be due to an artefact of the low sample size of juveniles in spring compared to the summer months.

Variation between sexes

Total leukocyte count was lower in males than in females. Males had lower lymphocyte, heterophil, H/L ratio, and eosinophils than females, but higher monocytes and basophils. However, adult males had higher lymphocyte counts than females. Males had lower RBC, PCV, and Hb than females. These findings are in accordance with results that showed that female great tits had higher H/L ratio and PCV than males (Ots *et al.*, 1998), however, the same study had found that females had higher prevalence of *Haemoproteus* spp. infection than males, hence their haematological values may have reflected a response to parasitism. Another study on great tits also found that the H/L ratio and PCV were higher in females than in males (Kilgas *et al.*, 2006), although a study on barn swallows found that males had higher PCV than females, but the difference between the sexes disappeared once difference in tail length was controlled for (Saino *et al.*, 1997). A study on kestrel nestlings (*Falco tinnunculus*) found no difference in PCV between sexes (Martinez-Padilla *et al.* 2004), suggesting that the differences between males and females may arise after fledging, although evidence of this is lacking (Fair *et al.*, 2007), and some studies have found no differences between the sexes (Fairbrother and O'Loughling, 1990).

Contrary to what is reported in the literature (Samour and Howlett, 2008), this study showed higher fibrinogen quantity in males than in females. If this was due to an ongoing infection, the result would be mirrored in the H/L ratio, but the latter was

lower in males than females. The difference between sexes remains, therefore, largely unexplained.

Variation with condition

Total leukocyte count was not correlated with body condition, in accordance with a study on great tits that found no correlation between body weight and total leukocyte count (Hauptmanová *et al.*, 2002). Lymphocytes were positively correlated with condition, while heterophils were negatively correlated with condition overall and in adults alone, which explain the negative correlation between condition and H/L ratio. In juveniles, however, heterophil count was positively correlated with condition. These results were in contrast with a previous study that had found no correlation between condition and heterophil, lymphocyte and eosinophil percentages (Fokidis *et al.*, 2008).

Monocyte count was positively correlated with condition, while basophils and eosinophils were negatively correlated with body condition. The H/L ratio can be used as an indicator of stress (Maxwell, 1993; Gross and Siegel, 1983), because heterophils increase and lymphocytes decrease in response to stressors (Samour and Howlett, 2008). Condition of the birds was negatively correlated with stress response, as indicated by the negative correlation with the H/L ratio in both adults and juveniles, and lower eosinophils, also known to decrease with stress (Jain, 1986). In a study on great tits, however, no correlation was found between body weight of healthy individuals and leukocyte differential count (Hauptmanová *et al.*, 2002).

Total erythrocytes and PCV were not correlated with condition, in accordance with some studies (Fokidis *et* al., 2008; Hauptmanová *et al.*, 2002; Villegas *et al.*, 2002; Dawson and Bortolotti, 1997a;b), although in a study on nestling barn swallow the authors found a weak correlation between haematocrit and condition (Cuervo *et al.*, 2007) . Hb showed a negative correlation with this measurement, but not when only juveniles were considered. This result may support the idea that higher Hb values are associated with higher metabolic demands for oxygen (Davey *et* al., 2000; Hõrak *et al.*, 1998; Saino *et al.* 1997; Clemens, 1990; Jaeger and McGrath, 1974), since body condition decreases during protracted periods of energetic demands due to energetic stress (Merilä and Wiggins, 1997). A possible explanation for the absence of a

relationship between condition and Hb in juveniles may be due to lower Hb levels in young birds than in adults. The use of heamatocrit as proxi for body condition has been criticised (O'Brien *et al.*, 2001), and the use of Hb has been suggested instead as a better estimate of oxygen transportation potential. Fibrinogen did not vary with condition, a result in accordance with previous authors (Samour and Howlett, 2008; Jain, 1993).

Variation between breeding status

Breeding birds had lower lymphocyte and higher heterophil counts than non-breeding birds, and the H/L ratio was consequentially higher in breeding individuals than in non-breeding adults, indicating that there may be a stress component associated with the act of breeding. A similar result was found in great tits (Pap *et al.*, 2010). Ots and Hõrak (1996) found that great tits that made a higher effort during the breeding season had higher H/L ratio than birds that made a smaller energetic investment, while another study on great tits showed that males and females with experimentally enlarged broods had higher haematocrit and H/L ratio than control or reduced broods (Hõrak *et al.*, 1998).

Breeding birds also had higher monocyte, eosinophils and basophils than non-breeding birds. Haemoglobin was also higher in breeding birds than in non-breeding birds, a result in accordance with previous studies (Davey *et al.*, 2000), and possibly supporting the positive correlation between haemoglobin and oxygen demand due to increased workload (Davey *et* al., 2000; Hõrak *et al.*, 1998; Saino *et al.* 1997). Not enough data were available to test differences in the other haematological values between breeding and non-breeding birds.

Variation between moult status

Sample size of moulting adults was very small, hence results on moulting birds of this age class should not be generalised. Moulting birds had higher total leukocyte count than non-moulting birds, a result in contrast with another study on house sparrows which found that moulting did not affect WBC count (Nava *et al.*, 2001). Moulting adults had higher lymphocyte and lower heterophil, and consequently higher H/L ratio than non-moulting birds of the same age. Moulting juveniles had

lower lymphocytes, higher heterophils, and lower H/L ratio than non-moulting juveniles.

Moulting adults had lower monocytes than non-moulting individuals, but moulting juveniles had higher monocyte count than non-moulting juveniles. Eosinophils were lower in moulting adults than in non-moulting ones, but they were higher in moulting juveniles than in non-moulting ones. Basophils were lower in moulting birds, adults and juveniles, than in non-moulting individuals. Nava and colleagues (Nava *et al.*, 2001) found that moult in house sparrow in both age groups did not affect lymphocyte nor eosinophil counts, but it increased the number of monocytes and basophils. A study on wild house finches showed that moulting birds had higher eosinophils than non-moulting birds (Davis *et al.*, 2004). Fairbrother and O'Lougling (1990) found a similar pattern in adult mallard to the one found in adults in this study, in which all leukocyte types decreased during moult apart from lymphocyte which increased. Driver (1981) found a similar pattern in mallard drakes, although total leukocyte count and lymphocyte count was also lower in moulting birds than in non-moulting individuals.

Moulting adults had lower total erythrocyte count but higher Hb than non-moulting individuals, but there were not enough data on adults during the moulting season to compare their PCV. Pap and colleagues (Pap *et al.*, 2010) found that haematocrit in great tits was at its lowest during the first part of the moulting season, while Driver (1981) found that total erythrocyte count, PCV, and Hb were lower in moulting adult males than in non-moulting birds. In this study, moulting juveniles had higher total erythrocyte count but lower PCV and Hb than non-moulting individuals.

This is a surprising results, because non-moulting juveniles are the youngest ones and they should have lower Hb, which is known to increases with age, as shown in this and other studies (Simmons and Lill, 2006; Shapiro *et al.*, 1999; Kostelecka-Myrcha *et* al., 1997; Merino and Barbosa, 1997; Puerta *et al.*, 1995). After about six weeks after fledging, juveniles start their post-juvenile moult (Ginn and Melville, 1983) at the end of which they will be indiscernible from adults. Juveniles that are not moulting are therefore younger than the moulting juveniles. The higher Hb of non-moulting juveniles is, however, matched by a higher PCV in non-moulting

juveniles, which can therefore indicate dehydration or a response to a stress stimulus (Samour and Howlett, 2008). Another possible explanation for the opposite trend between moulting adults and juveniles may be the age-confounding factor associated with non-moulting juveniles, which are younger birds, and hence potentially more prone to stress associated with reduced ability to forage, (Goss-Custard and Le V. Dit Durrell, 1987; Sutherland *et al.*, 1986) and avoiding predators (Geer, 1982), typical of juvenile birds in the early post-fledging period. Moulting adults had lower fibrinogen than non-moulting birds, but moulting juveniles had higher fibrinogen than non-moulting juveniles

3.5.3 Enteric bacteriology

Prevalence of *Salmonella* spp. and *Escherichia coli* was low in this study. None of the 406 swabs tested positive for *Salmonella* spp. or *E. coli,* and only 1 of the 271 live house sparrows tested positive for *Salmonella* spp.. This prevalence was similar to that found in a previous studies, where the authors had found 0% prevalence of *Salmonella* spp. and 1% *E. coli* using cloacal swabs of 364 apparently-healthy passerines (Brittingham *et al.*, 1988), and 5% *E.coli* prevalence was found in 98 apparently-healthy birds (Glunder, 1981), despite bird feeding stations having been considered a vehicle of transmission of pathogens between birds (Pennycott *et al.*, 2002; Locke *et al.*, 1973). Refsum and colleagues (Refsum *et al.*, 2003) found that in healthy-looking house sparrows prevalence of *Salmonella* was 8%, but his study was based on a small sample size of 25 individuals. The prevalence of positive cases increases if dead or sick birds are tested. For example 15% of healthy-looking house sparrows tested positive for *Salmonella* Thyphimurium in Canada compared to 90% of dead individuals of the same species submitted for post mortem (Tizard *et al.*, 1979; Tizard, 1994). The current study had focused on apparently healthy birds, so the result should be taken primarily in the context of surveillance.

A previous study on bird table (Pinches, 2002) had found similar prevalence of pathogens, indicating that the overall presence of *Salmonella* spp., may be quite low in bird tables. A study on faeces at bird feeders used by birds throughout the year found a prevalence of *Salmonella* spp. of up to 48% (Pennycott *et al.*, 2002), but another study found low prevalence in a feeder used only in winter (Pennycott *et al.*,

1998). The absence of positive feeder swabs may be due in part to a methodological bias. The present study relied on the help of garden owners that were enthusiastic enough to take part in the project, and may be more aware of the bird feeder hygiene procedures than the average garden owner.

Previous studies on wild birds, especially gulls (Literak *et al.*, 1992 ; Quessy and Messier, 1992) had found that the prevalence of *Salmonella* sp Typhimurium was higher than the one found in this study. One possible reason behind the different result is the different sampling method and habitat. Gulls are usually trapped on waste collection cites in big cities (Tizard, 1994), where in addition to general contact between food and waste, the birds also share the habitat with rodents, in particular rats (*Rattus* spp.) which can carry several pathogens including *Salmonella* spp. (e.g. Weisbroth *et al.*, 2006). It is perhaps interesting to report that the only sparrow that tested positive for *Salmonella* sp Typhimurium came from a site where the owner had seen (and controlled) rats (Clegg, *pers. comm.*.).

The low prevalence of birds with *Salmonella* spp. and *E. coli* spp. may also be due to a bias in the sampling methodology. Birds infected with *Salmonella* spp. are lethargic, fluffed up, and generally less mobile than healthy birds (Daoust and Prescott, 2007). It is presumable that birds with *Salmonella* spp infection, because they are less mobile, are also less likely to be caught in a mist-net, which is the method of capture used in this study. This bias may partially explain the low prevalence of infected birds found in this study, which agrees with previous authors that found that prevalence of *Salmonella* spp. and *E. coli* infections was very low in apparently healthy wild birds (Brittingham *et al.*, 1988; Goodchild and Tucker, 1968).

3.5.4 Haemoparasites

The haemoparasite present were *Atoxoplasma* spp, *Plasmodium* spp, *Leucocytozoon* spp, and microfilariae. The two most noticeable genera missing were *Haemoproteus* and *Trypanozoan,* which are commonly found in wild passerines (Merino *et al.*, 2000; Dale *et al.*, 1996), and *Haemoproteus* spp in particular are considered the most prevalent in wild passerines (Atkinson and Van Riper, 1991). Leucocytozoon was

reported in 100% of tawny owls (*Strix aluco*) in a study in the UK (Appleby *et al.*, 1999). Previous authors had also found no evidence of *Haemoproteus* spp nor *Trypanosoma* spp. (Fokidis *et al.*, 2008), while others have found *Trypanozoan* spp in wild birds in prevalence below 10% (Garvin *et al.*, 2003a), between 10-20% (Sanz *et al.*, 2002; Sanz *et al.*, 2001b; Siikamäki *et al.*, 1997; Dale *et al.*, 1996), or ranging 20-30% (Wiehn and Korpimäki, 1998). About 10% of individuals in a population will be positive for at least one species of haematozoa, and their prevalence can range from 0 to 100% depending in the host species and ecological factors (Janovy, 1997).

Prevalence of *Haemoproteus* spp. varies according to species and study. In adult house martins it was 48.3% (Marzal *et al.*, 2005) in one study, and between 43.2% and 47.1% in another one (Christe *et al.* 2002). In pied flycatchers it was reported to be between 10-40% (Siikamäki *et al.*, 1997), 48.3% (Dale *et al.*, 1996), and between 16% and 35% but only in females (Sanz *et al.*; 2001; Sanz *et al.*, 2001b). In great tits, researchers found, for example, 50.9% prevalence (Allander, 1997), and 75.7% (Allander and Bennett, 1994). In American redstarts (*Setophaga ruticilla*) prevalence was 5% (Garvin *et al.*, 2004), 40.8% in gray catbirds (*Dumatella carolinensis*) (Garvin *et al,* 2003(a)), 43.2% in house sparrows (Navarro *et al.* , 2003), between 0-80% in red-bellied woodpeckers (Schrader *et al.*, 2003), 9% in dark-eyed juncos (*Junco hyemalis*) (Deviche *et* al., 2001), 27% in European kestrels (Wiehn and Korpimäki, 1998), and up to 42% in purple martins (*Progne subis*) (Davidar and Morton, 1993).

Plasmodium spp.

The total prevalence of *Plasmodium* spp. was 21.8%, and 29.7% in adults and 14.4% in juveniles. The overall prevalence of this haemoparasite varies from species to species (e.g. Deviche *et al.*, 2001). Prevalence of *Plasmodium* spp infection is very variable. Allander and Bennet (1994) found 4.8% prevalence in great tits in Sweden, and Allander (1997) found 5.5% prevalence in the same species and area. Adults had higher prevalence than juveniles, a result in accordance with previous findings on haemoparasites in other passerines (Sanz *et al.,* 2001b; Dale *et al.*, 1996; Davidar and Morton, 1993; Wakelin, 1984) and waders (Mendes *et al.*, 2005), possibly due to a relapse of a latent infection (Sanz *et al.*, 2001b), or adults having longer exposure

to vectors and hence more time to acquire parasites than juvenile birds (Allander and Bennett, 1994; Davidar and Morton, 1993). Difference between ages was not found, however, by all authors (Allander, 1997).

The prevalence of *Plasmodium* did not vary between sites, which may mean that birds from all sites may have the same chance of being bitten by an infected vector. The intensity of *Plasmodium* infection, however, was significantly different across sites, which may suggest that birds in some sites are less able to control the infection, since all sites were sampled at the same time intervals so there cannot be an effect of season on the intensity of infection (see chapter 4).

Infected birds surprisingly had lower total leucocyte counts than non-infected birds, and leucocytes also decreased with intensity of infection. These results are in contrast with findings from previous authors that had found a positive association between infection by haemoparasite and high total leucocyte counts, both with *Plasmodium* spp infections (Figuerola *et al.*, 1999), and *Haemoproteus* spp. infections (Ots and Hõrak, 1998).

Infected birds had a higher H/L ratio than non-infected birds, suggesting that *Plasmodium* parasites had triggered an immune response in infected birds. Previous studies also found increasing H/L ratio in relation to haemoparasite infection (Fokidis *et al.*, 2008; Ots and Hõrak, 1998), although the parasites considered were *Haemoproteus* spp. and *Trypanosoma* spp. When only infected birds were considered, the H/L ratio decreased with increasing intensity of the infection, reflecting the increased number of heterophils and the lower lymphocyte count as infection intensity increases. These results were in contrast with a previous study on great tits in which lymphocyte number, but not heterophils, increased with the intensity of infection (Ots and Hõrak, 1998), and with a study on cirl bunting that found higher lymphocyte and lower heterophil counts in birds with *Plasmodium* spp infection (Figuerola *et al.*, 1999).

Eosinophils also increased in parasitised birds and with intensity of infection. A study on captive blue jays (*Cyanocitta cristata*) experimentally infected with *Haemoproteus* also found higher counts of all leukocytes, including eosinophils, in

infected birds compared to control individuals (Garvin *et al.*, 2003). Eosinophils are thought to react specifically to parasites (Samour and Howlett, 2008; Maxwell, 1987), and they can be used to differentiate an increase in WBC and H/L ratio due to parasites, from that due to stress (Davis *et al.*, 2008), which decreases the number of eosinophils (Jain, 1986). Increased number of eosinophils associated with *Plasmodium* found in this study may suggest that parasites had triggered an immune response.

Monocyte count was lower in infected birds, but it increased with intensity of the infection. Contrary to this study, work on captive blue jays had found higher monocytes count in birds infected with *Haemoproteus* (Garvin *et al.*, 2003). This type of leukocyte responds to chronic infections (D'Aloia *et al.*, 1994) but not specifically to parasites, so changes in monocytes count associated with *Plasmodium* prevalence or intensity of infection are unexpected.

Values that indicate number or volume of erythrocytes (total RBC and PCV) were higher in infected birds, and they increased with intensity of the infection. These results seem counter-intuitive when considering that *Plasmodium* is known to destroy erythrocytes (Atkinson and Van Riper III, 1991), and it should be associated with anaemia (Atkinson, 2008; Atkinson and Van Riper III, 1991). However, both RBC and PCV values cannot differentiate between mature erythrocytes and immature ones (rubricytes) which carry significantly less haemoglobin and hence oxygen than mature red blood cells (Jain, 1993).

In recent years the validity of total erythrocyte and PCV has been questioned in favour of the values of Hb present in the blood as a better estimate of the damage of the parasites to the circulating erythrocytes (O'Brien *et al.*, 2001). In this study the amount of Hb, its average content in single erythrocytes (MCH), and the volume occupied by Hb within the erythrocyte (MCHC) were lower in birds infected with *Plasmodium* spp, and the three values decreased with the intensity of the infection. This may indicate that the parasitised individuals are replacing the lost erythrocytes with immature ones which contain less haemoglobin, and hence the parasite is causing at least a transient damage to the host.

3.5.5 Gastro-intestinal parasites

Isospora spp.

The total prevalence of *Isospora* parasites was 31.4%, 27.2% in adults and 34.5% in juveniles. The higher prevalence in juveniles than in adults is in accordance with previous studies (Greiner, 2008; Novilla and Carpenter, 2004) which have found that coccidian parasites are more frequently found in young birds, although the author suggested that adults are usually not affected (Greiner, 2008), while others have found that intensity, rather than prevalence, was higher in juveniles than in adults (Lopez *et al.*, 2007). In a previous study on two populations of robins (*Erithacus rubecula*) in New Zealand, prevalence was 2-3% (Hale and Briskie, 2007). In 135 house sparrows (subspecies *biblicus*) sampled in Israel prevalence ranged from 51.6% to 68% (Gill and Paperna, 2008), and natural *Isospora* spp. prevalence was 89% in 25 greenfinches (*Carduelis chloris*) before they were taken into captivity (Hõrak *et al.*, 2004).

The intensity of the infection varies greatly from individual to individual (Dolnik, 2006; Hõrak *et al.*, 2006), and with the time of the day, with peak shedding of oocysts occurring in the late afternoon (Filipiak *et al.*, 2009; Martinaud *et al.*, 2009; López *et al.*, 2007; Brown *et al.*, 2001; Hudman *et al.*, 2000; Brawner and Hill, 1999; Boughton, 1933). Unfortunately, in the current study it was not possible to sample birds in the afternoon for two main reasons: first, mist-netting of birds is more successful early in the morning, when bird activity is at its peak, and second, because the fresh blood sample had to be processed on the same day (Samour and Howlett, 2008; Samour, 2006), and each sample took an hour to process, so the sampling time in the day was constrained by these two factors. Sampling was, however, consistently done early in the morning, so the results from the different birds are comparable.

Birds shedding oocysts had a higher leucocyte count than birds that were not shedding oocysts, indicating that the immune system had reacted to the infection, as indicated by the higher H/L ratio found in infected birds. Previous authors reported heterophilia associated with infection of *Isospora* spp in greenfinches (Hõrak *et al.*, 2004), and changes in lymphocyte numbers and associated immune response in Kestrels (*Falco tinnunculus*) in response to coccidian infection (Lemus *et al.*, 2010).

Eosinophil count was lower in infected birds than in non-infected birds, which is surprising since this type of leucocyte increases in number when there is a parasitic infection. However, it is possible that the shedding of oocyst at this time of the day may be more indicative of the intensity of infection, and only heavily parasitised birds shed oocysts early in the morning. Monocyte count was higher in individuals shedding *Isospora* spp. oocysts, suggesting that the infection may be chronic, since monocytes increase with this type of infections (D'Aloia *et al.*,1994). Dolnik (2006) suggested that most birds in the wild probably have a chronic infection (Dolnik, 2006). The pathogeneity of *Isospora* spp. parasites is still under debate (Greiner, 2008), but while it may not develop in all cases into a clinical disease, it is still plausible that the immune system reacts to the parasite.

The total erythrocyte count, Hb and PCV values were higher in birds shedding *Isospora* than in birds not shedding the parasites. This result may be surprising, but relative polycythemia may also be due to dehydration, especially in diseases, such as coccidiosis, accompanied by excessive loss of water or malabsorption (Jain, 1993). Firbinogen quantity was higher in individuals shedding oocysts than in those that did not, indicating that the organism had initiated an inflammatory response, as suggested also by the increased H/L ratio.

Atoxoplasma spp.

The overall prevalence of *Atoxoplasma* spp was 30%, 28% in adults and 31.2% in juveniles. The prevalence of this parasite varies greatly between species and between studies. In a study on Hawaian birds, it ranged from 0.1% to 17.4% according to the species, with 8.6% of house sparrows testing positive (Van Riper III *et al.*, 1987), while in Poland the prevalence was up to 100% in 90 tree sparrows and house sparrows (Kruszewicz, 1991), but only 2.9% over 4,070 house sparrows in Canada (Greiner *et al.*, 1975). Adults and juveniles did not differ in prevalence, which is in contrast with higher prevalence in juveniles than adults suggested by some authors (Greiner, 2008; Pierce, 2008).

Infected birds had higher total leukocyte count, which increased with intensity of the infection, suggesting that the parasite had stimulated the immune system. Heterophils and lymphocytes count were higher in parasitised individuals, but the H/L ratio was

lower in infected birds. Heterophils increased and lymphocyte decreased with the intensity of the infection, and consequentially H/L ratio increased with intensity of parasitaemia, as expected in the presence of infection (Samour and Howlett, 2008).

Monocyte count was lower in infected birds and it decreased with infection rate. This result is not surprising considering that *Atoxoplasma* in this study infected primarily monocytes, and these parasites are known to eventually kill their host cell (Greiner, 2008). The presence of *Atoxoplasma* presumably reduced the number of circulating monocytes. Eosinophil count was higher in parasitised individuals and it increased with intensity of infection, which is in accordance with the parasitic-specific response that this type of leucocyte is involved in (Campbell and Ellis, 2007; Maxwell, 1987), and it is suggestive of a stimulation of an immune response against *Atoxoplasma* infection.

Total erythrocyte counts and Hb were lower in parasitised birds and they decreased with infection rate. This result is unexpected, because this parasite is not known to have a detrimental effect on erythrocytes. The PCV and MCV were higher in parasitised individuals and they increased with intensity. A possible explanation is that *Atoxoplasma,* as a pathogen thought to originate from coccidian parasites that are linked to reduced absorption of water and nutrients (Greiner, 2008), may be linked to dehydration, which is turn causes haemoconcentration and higher haematocrit values (Samour, 2006).

Fibrinogen quantity was also higher in infected birds compared to non-infected birds, although it did not vary with intensity of the infection. Fibrinogen increases in cases of infection (Samour and Howlett, 2008), but the amount of fibrinogen was not correlated to the intensity of the infection, which is a result in accordance with previous authors (Jain, 1993).

3.6 CONCLUSIONS

Basal haematological values of some leucocytes were higher than the normal ranges suggested in veterinary reference books. However, reference values are usually based on captive animals, and on different species. Previous studies on house sparrow

haematology were either based on a small sample size, a restricted period of the year, or on animals that had not been checked for infection of pathogens.

In this study, haematological values of pathogen-free house sparrows showed variation between seasons, ages, sexes, condition, breeding status, and moult status expected considering the physiological changes associated with these variables. Infection by *Plasmodium* spp., *Atoxoplasma* spp., and *Isospora* spp. were associated with changes in leucocyte- and erythrocyte-related values, indicating that the presence of these parasites elicited a physiological response in its host.

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Chapter 4

PARASITE PREVALENCE AND INTENSITY OF INFECTION VARIATION WITH SEASON, AGE, SEX, CONDITION OF THE HOST, BREEDING STATUS, AND MOULT

4. PARASITE PREVALENCE AND INTENSITY OF INFECTION - VARIATION WITH SEASON, AGE, SEX, CONDITION OF THE HOST, BREEDING STATUS, AND MOULT.

4.1 INTRODUCTION

Variation of parasites between years depends on several factors, including the population size of the host and of any vectors involved, the number of susceptible hosts (including vectors), the pathogenicity of the parasite, on other characteristics of the biology and behaviour of the host (and vector) (Schall and Marghoob, 1995), and the ecology of vectors involved (Wood *et al.*, 2007). Lack of variation in parasite distribution between years may reveal spatially-dependent host-parasite co-evolution (Wood *et al.*, 2007), but any interpretation should consider the cyclic pattern of the disease. Malaria, for example, seems to have a 10 year cycle in different species, including humans (Schall and Marghoob, 1995; Forsyth *et al.*, 1989).

Seasonal variation in parasite prevalence and intensity can be the result of variation in both host physiology (e.g. immune system), and exposure of the host to infective stages of the parasite (Wilson *et al.*, 2002). Seasonality in haematozoa prevalence is well documented, and it varies between temperate and tropical zones (Atkinson and van Riper, 1991). Prevalence of infection often peaks in spring (Schrader *et al.*, 2003; Garvin *et al.*, 2003; Hatchwell *et al.* 2000), termed the "spring relapse" (Box, 1966), due to changes in corticosterone levels associated with the onset of the breeding activity (Applegate, 1970; Applegate and Beaudoin, 1970), increased day-length, and stress (Valkiūnas *et al.*, 2004). A second peak is often recorded in late summer and beginning of autumn (Cosgrove *et al.*, 2008; Ricklefs and Sheldon, 2007; Garvin *et al.*, 2003; Schrader *et al.*, 2003), when the number of mosquito vectors is high (Cranston *et al.*, 1987), and juveniles born the same year provide immunologically-naïve hosts (Altizer *et* al., 2006; Beaudoin *et al.*, 1971).

Differences in prevalence and intensity of parasites between age classes may be due to different exposure to parasites or their vectors (Wilson *et al.*, 2002), and lack of acquired immunity in juveniles (Altizer *et* al., 2006; Beaudoin *et al.*, 1971). Several studies on avian species have reported higher levels of haematozoan parasites in juveniles than in adults (Cosgrove *et al.*, 2008; Wood *et al.*, 2007; Valkiūnas *et al.*, 2006; Mendes *et al.*, 2005; Sol *et al.*, 2003; Waldenström *et al.*, 2002; Sanz *et al.*, 2001b; Dale *et al.*, 1996; Allander and Bennett, 1994; Norris *et al.*, 1994; Davidar and Morton, 1993); however, several authors have not found a difference in prevalence of haematozoan between ages (Evans *et al.*, 2009; Garvin *et al.*, 2003; Figuerola *et al.*, 1999; Allander, 1997; Van Riper *et al.*, 1986). Contrasting results have also been found in levels of *Isospora* spp. parasite infection between ages, with some authors finding higher prevalence in young birds (Valkiūnas *et al.*, 2006; Kozłowski *et al*, 1991), but others failing to find such variation (Lopez *et al.*, 2007; Hudman *et al.*, 2000); *Atoxoplasma* spp. are thought to have higher infection in young birds (Greiner, 2008), but strong support and experimental evidence are lacking.

Sex-biased parasitism has long been recorded in animals, and males have higher parasite prevalence and intensity of parasitism in several taxa, due to differences in behaviour and physiology (Klein, 2004). In birds, prevalence of nematodes was found to be higher in males than females (Poulin, 1966), but most studies that found a difference between sexes indicate that haematozoan levels are higher in females (McCurdy *et al.*, 1998; Ots *et al.* 1998; Norris *et al.*, 1994; Applegate *et al.*, 1971), although some found higher prevalence in males (Wood *et al.*, 2007), and others found no correlation (Cosgrove *et al.*, 2008; Martin *et al.*, 2007; Schrader *et al.*, 2003; Christe *et al.*, 2002; Figuerola *et al.*, 1999; Gonzalez *et al.*, 1999; Ots and Hõrak, 1998; Allander, 1997; Dale *et al.*, 1996; Allander and Bennett, 1994; Davidar and Morton, 1993). Contrasting results have also been found for differences of *Isospora* spp. between sexes: some authors have found higher prevalence and intensity in females (Aguilar *et al.*, 2008), and others did not find differences between males and females (Filipak *et al.*, 2009).

Parasitic infections are costly to their host (Goater and Holmes, 1997; Møller *et al.*,1990), not only because, by definition, parasites absorb energy from it (Clayton and Moore, 1997), but also because controlling the infection is energetically costly from the immunological point of view (Klasing *et al.*, 1991 cited in Wakelin and Apanius, 1997). Birds parasitised may, therefore, show decreased body condition as they use energy reserved to fight the infection. Some authors have found no correlation between level of haematozoan parasitism and body condition (Palinauskas *et al.*, 2008; Martin *et al.*, 2007; Kilpatrick *et al.*, 2006; Navarro *et al.*, 2004; Sol *et al.*, 2003; Christe *et al.*, 2002; Sanz *et al.*, 2001; Figuerola *et al.*, 1999; Gonzalez *et al.*, 1999; Bennett *et al.*, 1998), but others have found reduced body weight in birds infected with haematozoan (Schrader *et al.*, 2003; Merino *et al.*, 2000; Dawson and Bortolotti, 2000), although in some cases loss of body mass was mainly due to reduced feeding rate of the infected individuals (Atkinson *et al.*, 1995).

Infection by *Isospora* spp. have mostly been associated with loss of body mass (Aguiler *et al.*, 2008; Costa and Macedo, 2005; Hõrak *et al.*, 2004), although some studies only reported a transient effect (Pap *et al.*, 2009), or loss of body mass associated with reduced food intake (McGraw and Hill, 2000). However, some studies have failed to find any effect between *Isospora* spp. parasitism and changes in body mass (Baeta *et al.*, 2008; Dale *et al.*, 1996).

Reproduction is costly, and an individual faces a trade-off between current and future reproductive success (Williams, 1966). Resources available to an individual are finite, and a further trade-off has been suggested between energy allocated to reproduction and energy allocated to fighting infections by parasites (Gustaffson *et al.* 1994).

A trade-off in birds between the ability to fight haematozoan infections and the breeding process has been suggested through results of observational studies (Sanz *et al.*, 2001; Allander and Bennett, 1995; Van Riper *et al.*, 1986), brood manipulation experiments (Knowles *et al.*, 2010b; Nordling *et al.*, 1998; Siikamäki *et al.*, 1997; Oppliger *et al.*, 1996; Richner *et al.*, 1995; Norris *et al.*, 1994), and medication experiments aimed at clearing the infection (Knowles *et al.*, 2010a; Marzal *et al.*,

2005). Observational studies have shown an association between haematozoan prevalence and delayed egg-laying (Allander and Bennett, 1995), reduced hatching success (Sanz *et al.*, 2001), and increased desertion rate (Sanz *et al.*, 2001). However, some studies failed to find a negative correlation between haematozoan prevalence and parental and nestling performance (Szöllősi *et al.*, 2009; Kilpatrick *et al.*, 2006; Siikamäki *et al.*, 1997; Davidar and Morton, 1993), or in prevalence and intensity of haematozoan between breeding and non-breeding birds (McCurdy *et al.*, 1998; Van Riper *et al.*, 1986).

Experimental studies can help extricating cause and effect. Brood manipulation experiments have shown an association between increased breeding effort and increased prevalence and/or intensity of haematozoan in females (Nordling *et al.*, 1998; Oppliger *et al.*, 1996; Ots and Hõrak, 1996), males (Siikamäki *et al.*, 1997; Richner *et al.*, 1995; Norris *et al.*, 1994), or both sexes (Knowles *et al.*, 2010b; Knowles *et al.*, 2009). Medicational experiment found that medicated parents had reduced prevalence and intensity of haematozoan, and increased hatching, and fledging success than untreated parents (Knowles *et al.*, 2010a; Marzal *et al.*, 2005; Merino *et al.*, 2000).

Moult is an energy-demanding activity (Dawson *et al.*, 2000; Murphy and King, 1992), and experiments have shown that feather growth or quality were impaired by other concomitant energy-demanding activities, such as fighting coccidian infections (Hõrak *et al.*, 2004), and reproductive effort (Merilä and Wiggins, 1997; Siikamäki *et al.*, 1994). Experiments have also shown slower moulting time in birds mounting an immune response to phytohaemaglutinin (PHA) stimulation, and a lower response to PHA in moulting birds (Moreno-Rueda, 2010). However, one measure of immune response does not necessarily correlate with response to any other types of challenges (Adamo, 2004), therefore one cannot extrapolate a general ability to respond to parasite infections based on PHA response. However, trade-off between moult and mounting an immune response to a foreign antigen is probably applicable to other systems.

4.2 AIMS

The aim of this study were:

1. To compare prevalence and intensity of *Plasmodium* spp., *Atoxoplasma* spp., and *Isospora* spp. between years and seasons.
2. To compare prevalence and intensity of *Plasmodium* spp., *Atoxoplasma* spp., and *Isospora* spp. with age, sex, breeding status, moult, and condition of their hosts.

4.3 METHODOLOGY

4.3.1 Description of the variables

Parasite prevalence and intensity

Parasite counts were obtained following the methodology outlined in section 2.3.1. Parasites genera were considered separately. Prevalence of infection indicated the percentage of birds infected, while intensity indicated the level of the infection of the parasitised individuals.

Year and Season

Parasite data were available for three years: 2007, 2008, and 2009.

Seasons were defined as: spring (March to May), summer (June to August), autumn (September to November), and winter (December to February). This subdivision was chosen to match the season in the calendar, but each season was shifted backwards almost by one month (e.g. spring starts officially on 21 March), to match the biology of the species. Spring can be defined, in temperate zones where sites considered in this study are located, broadly as the season of nest-building, egg-laying and incubation, and early broods; summer is the peak of the breeding season, with second and third broods (Summers-Smith, 1963); autumn is the period directly after the end of the breeding season; finally, winter is the period of coldest weather.

Age

Age of house sparrows was ascertained using plumage description described by Svensson (Svensson, 1992). For further details on the problem of ageing house sparrow, please refer to section 2.2.1. For analyses between ages, which do not differentiate between sexes, the data had come from pooled sample of males and females.

Sex

Sexing of birds was done on plumage (Svensson, 1992). Juveniles are monomorphic and resemble adult females until they begin their post-juvenile moult. For details on sexing house sparrows please refer to section 2.2.1. For analyses between sexes, whcih do not differentiate between ages, the data had come from pooled sample of adults and juveniles.

Body condition

Condition was calculated as the residuals of the regression of body mass against tarsus length, to control for the structural size of the birds.

Breeding status

Breeding status was considered positive when females had a brood patch and males showed evidence of a cloacal protuberance. Birds that did not show a brood patch or a cloacal protuberance in the months of the breeding season (April to August) and that were not moulting were considered non-breeding.

Moult

Moulting period was considered to extend between July and end of September (Ginn and Melville, 1983). Adults were considered not moulting if during that period they were not showing signs of active remiges moult nor breeding [since house sparrows do not usually initiate moult until after breeding is complete (Anderson, 2006)].

4.3.2 Statistical analyses

All analyses were conducted in R 2.11.1 (R Development Core team, 2010) using Tinn-R R (Development Core team, 2010) as text interface.

The significance of each explanatory variable was calculated by comparing the model with the variable to a null model using the likelihood ratio test, as suggested by Douglas Bates (2006). The p-values reported in the results are those of the likelihood ratio test specifying the F distribution to control for overdispersion.

Prevalence and intensity were analysed separately. The prevalence and intensity of infection of each parasite genus was compared between years, seasons, ages, sexes, breeding status, moult, and body condition. The interactions between sex and age, sex and condition, age and condition, and moult and age were also investigated.

Mixed Effect Models were applied using "site" as random effect. For prevalence data, the error family was specified as quasibinomial to account for overdispersion, and for intensity data a quasipoisson error structure was specified, because counts of parasites contained integers, but they were overdispersed. Since the dispersion parameter was larger than 1, the significance of the parameters was tested with an F test (Crawley, 1993) .

Table 4.1. Prevalence and mean intensity of *Plasmodium* spp., *Atoxoplasma* spp., and *Isospora* spp. across the three years of the study.

Parasite	**Year**		
	2007	2008	2009
Plasmodium Prevalence	16.18% (28/173)	25.62% (41/160)	29.78% (14/47)
Plasmodium Intensity	25.03	40.56	8.57
Atoxoplasma Prevalence	36.47% (62/170)	23.89% (38/159)	27.65% (13/47)
Atoxoplasma Intensity	2.69	2.63	1.53
Isospora Prevalence	14.68% (21/143)	7.46% (5/67)	0% (0/32)
Isospora Intensity	2.95	4.4	N/A

Table 4.2. Prevalence and mean intensity of *Plasmodium* spp., *Atoxoplasma* spp., and *Isospora* spp. across seasons.

Parasite	**Seasons**			
	Winter	Spring	Summer	Autumn
Plasmodium Prevalence	38.29% (18/47)	20.14% (27/134)	19.83% (24/121)	20.28% (14/69)
Plasmodium Intensity	21.55	32.70	32.04	31.71
Atoxoplasma Prevalence	21.73% (10/46)	31.91% (45/141)	28.09% (34/121)	35.29% (24/68)
Atoxoplasma Intensity	2.2	3.11	1.85	2.58
Isospora Prevalence	20.45% (9/44)	9.34% (10/107)	8.97%	0% (0/23)
Isospora Intensity	3.44	3.1	3.14	N/A

Table. 4.3. Prevalence and mean intensity of *Plasmodium* spp. , *Atoxoplasma* spp., and *Isospora* spp. between breeding (Y) and non-breeding (N) birds.

Parasite	**Breeding status**	
	Y	N
Plasmodium Prevalence	35.85% (19/53)	16.66% (1/6)
Plasmodium Intensity	39.21	3
Atoxoplasma Prevalence	26.92% (14/52)	16.66% (1/6)
Atoxoplasma Intensity	1.78	2
Isospora Prevalence	0% (0/37)	69.23% (9/13)
Isospora Intensity	N/A	3.44

Table. 4.4. Prevalence and mean intensity of *Plasmodium* spp. , *Atoxoplasma* spp., and *Isospora* spp. between adults and juveniles.

Parasite	**Age**	
	Adults	Juveniles
Plasmodium Prevalence	29.72% (55/185)	14.35% (28/195)
Plasmodium Intensity	16.06	35.33
Atoxoplasma Prevalence	28.02% (51/182)	31.2% (62/194)
Atoxoplasma Intensity	2.43	2.69
Isospora Prevalence	8.7% (9/103)	12.23% (17/139)
Isospora Intensity	3.44	3.11

Table. 4.5. Prevalence and mean intensity of *Plasmodium* spp., *Atoxoplasma* spp., and *Isospora* spp. between females and males.

Parasite	**Sex**	
	Females	Males
Plasmodium Prevalence	26.82% (33/123)	23.17% (38/164)
Plasmodium Intensity	36.26	29.05
Atoxoplasma Prevalence	36.06% (44/122)	22.83% (37/162)
Atoxoplasma Intensity	1.79	2.64
Isospora Prevalence	7.04% (5/71)	11% (12/109)
Isospora Intensity	3.4	3.33

Table. 4.6 Prevalence and mean intensity of *Plasmodium* spp. , *Atoxoplasma* spp., and *Isospora* spp. between moulting (Y) and non-moulting (N) birds.

Parasite	**Moult**	
	Y	N
Plasmodium Prevalence	19.26% (21/109)	22.22% (26/117)
Plasmodium Intensity	35.04	33.42
Atoxoplasma Prevalence	28.44% (31/109)	29.56% (34/115)
Atoxoplasma Intensity	2.06	2.64
Isospora Prevalence	11.39% (9/79)	9.52% (8/84)
Isospora Intensity	3.33	2.87

Table. 4.7. Sample size (number of birds) for prevalence and intensity of *Plasmodium* spp. , *Atoxoplasma* spp., and *Isospora* spp. for condition.

Parasite	Body condition
Plasmodium Prevalence	376
Plasmodium Intensity	83
Atoxoplasma Prevalence	376
Atoxoplasma Intensity	113
Isospora Prevalence	242
Isospora Intensity	26

4.4 RESULTS

4.4.1 Year

There was no variation between years in *Plasmodium* spp. prevalence (χ^2= 4.7, p=0.09, df=2), nor intensity (χ^2=3.15, p=0.07, df=1). There was no variation between years in *Atoxoplasma* spp. prevalence (χ^2=23.55, p=0.16, df=2), but intensity was almost significantly different overall (χ^2=3.65, p=0.055,df=1). There was no significant differences between years in *Isospora* spp. prevalence (χ^2=1.85, p=0.39, df=2) or intensity (χ^2=2.41, p=0.12, df=1).

4.4.2 Season

Plasmodium spp. prevalence did not vary overall across seasons (χ^2= 7.06, p=0.069, df=3), but intensity did (χ^2=90.89, p=0.0001, df=1). Prevalence was higher in winter than in summer (χ^2=5.62, p=0.017, df=1), higher in winter than spring (χ^2=6.21, p=0.012, df=1), and higher in winter than autumn (χ^2= 3.93, p=0.047, df=1). Intensity in summer was higher than in spring (χ^2=58.97, p=0.0001, df=1), in summer was higher than in winter (χ^2=83.25, p=0.0001, df=1), in autumn higher than

in spring (χ^2=83.62, p=0.0001,df=1), in winter was higher than in spring (χ^2=7.07, p=0.007, df=1), and in autumn higher than in winter (χ^2=60.53,p=0.0001, df=1).

Atoxoplasma spp. prevalence did not differ overall between seasons (χ^2=4.32, p=0.22, df=3), and none of the pairwise differences were significant. *Atoxoplasma* spp. intensity was almost significantly different between seasons (χ^2=7.66, p=0.053, df=3), and in pairwise comparisons autumn intensity was higher than in summer (χ^2= 4.19, p=0.0001, df=1), it was higher in winter than in summer (χ^2=_4.78, p=0.0001, df=1) , higher in spring than in autumn (χ^2=6.02, p=0.0001, df=1), and higher in spring than in winter (χ^2=6.27, p=0.0001, df=1) .

Isospora spp. prevalence differed overall between seasons (χ^2=12.28, p=0.006, df=3). In pairwise comparison prevalence was higher in summer than in autumn (χ^2=4.4, p=0.035, df=1), higher in winter than in summer (χ^2=4.1, p=0.042, df=1), higher in spring than in autumn (χ^2=4.07, p=0.043, df=1), higher in winter than in spring (χ^2=5.96, p=0.014, df=1), and higher in winter than in autumn (χ^2=10.98, p=0.0009, df=1). *Isospora* spp. intensity did not vary overall across seasons (χ^2=0.19, p=0.9, df=1), and pairwise comparison showed that intensity was higher in winter than in summer (χ^2=1.32, p=0.0001, df=1), and higher in winter than in spring (χ^2=2.16, p=0.001, df=1) . Due to low sample size it was not possible to test differences in prevalence between summer and autumn, autumn and spring, and autumn and winter.

4.4.3 Age

Adults had higher prevalence of *Plasmodium* spp. (χ^2=12, p=0.0005, df=1) but lower intensity (χ^2=5.89, p=0.015, df=1) than juveniles.

There was no difference between ages in *Atoxoplasma* spp. prevalence (χ^2=1.55, p=0.21, df=1), or intensity (χ^2=0.22, p=0.63, df=1), and a similar result was found for *Isospora* spp. prevalence (χ^2=1.24, p=0.26, df=1) and intensity (χ^2=0.19, p=0.66, df=1).

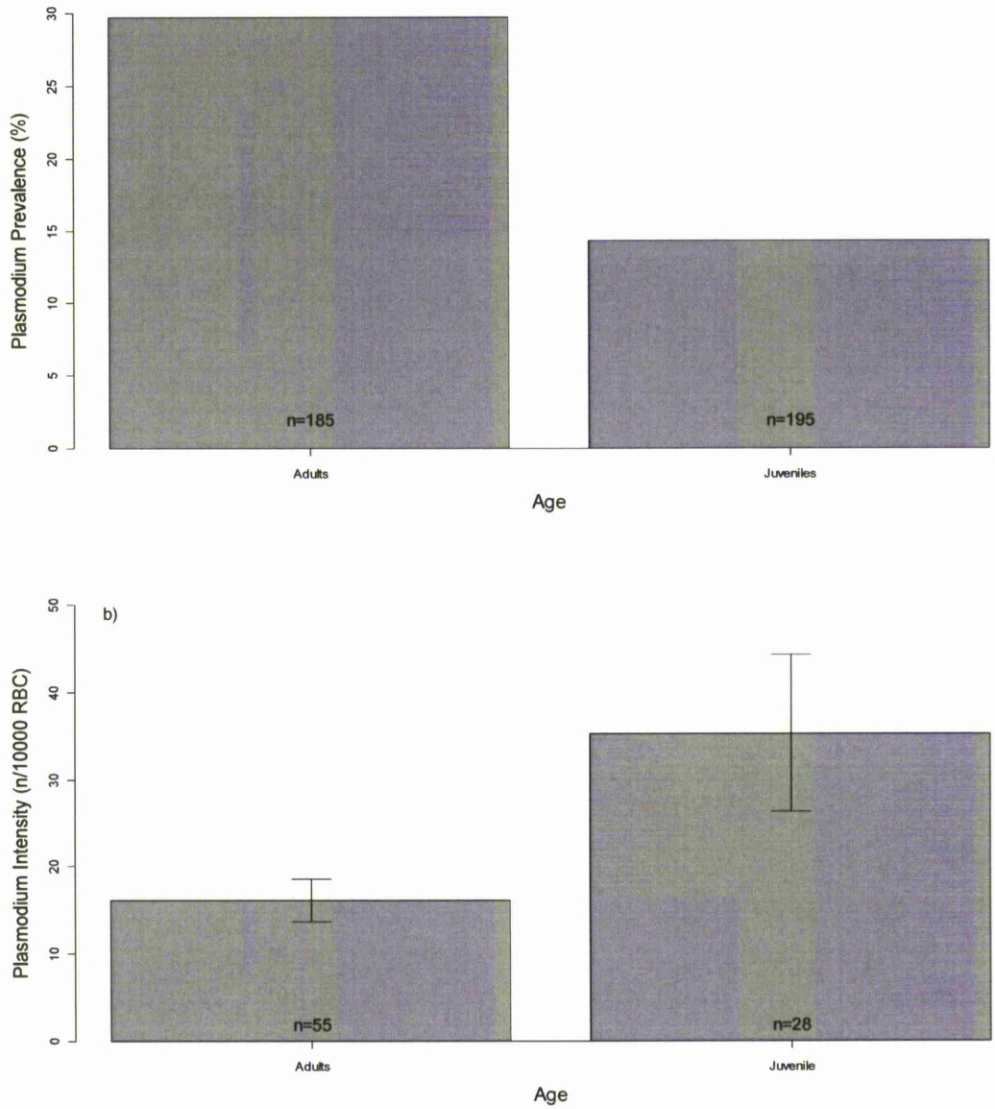

Fig. 4.1. Variation in *Plasmodium* spp. prevalence (a) and intensity (b) between adults and juveniles. Adults may have higher prevalence than juveniles because they have been exposed to vectors for longer. Juveniles have higher intensity of infection possibly because their immune system cannot control the infection as well as adults.

4.4.4 Sex

Males had lower prevalence of *Plasmodium* spp. (χ^2=77.38, p=0.00001, df=1) and lower intensity (χ^2=252.6, p=0.00001, df=1) than females. Males had lower prevalence of *Atoxoplasma* spp. (χ^2=118.84, p=0.00001, df=1) but higher intensity

(χ^2=114.65, p=0.00001, df=1) than females. Prevalence of *Isospora* spp. was higher in males (χ^2=55.47, p=0.00001, df=1), which however had lower intensity (χ^2=9.14, p=0.0024, df=1) than females. Age may be a confounding factor in differences between sexes, but the only significant interaction was with *Plasmodium* spp. intensity, which was lower in juvenile males than in juvenile females (χ^2=336.59, p=0.00001, df=1).

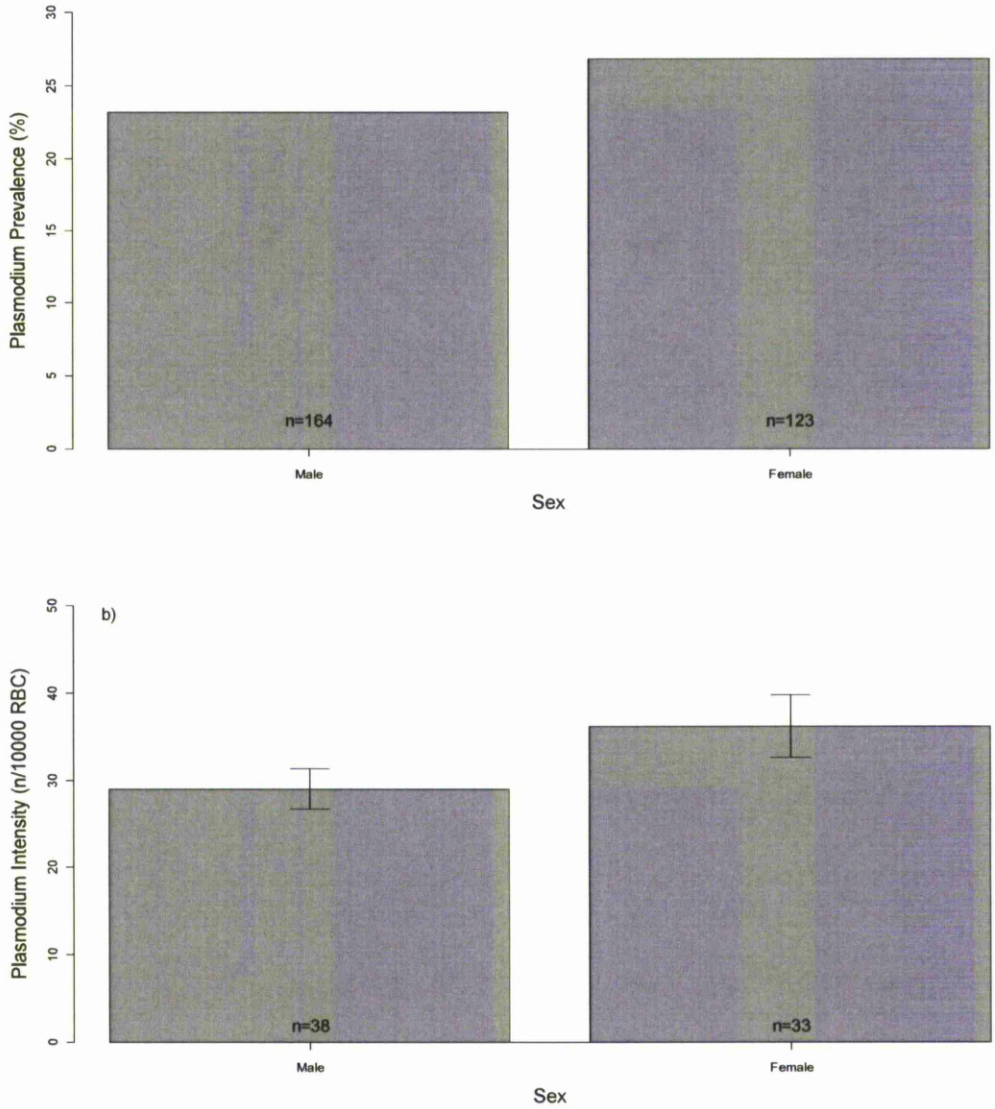

Fig. 4.2. Variation in *Plasmodium* spp. prevalence (a) and intensity (b) between males and females.

4.4.5 Body condition

Plasmodium spp. prevalence did not correlate with changes in condition (χ^2=0.15, p=0.69, df=1), but condition was negatively correlated with intensity of parasite (χ^2=14.22, p=0.0001, df=1). Condition was not correlated with *Atoxoplasma* spp. prevalence (1.71, p=0.19, df=1), nor intensity (χ^2=0.43, p=0.51, df=1), or *Isospora* spp. prevalence (χ^2=0.56, p=0.45, df=1) nor intensity (χ^2=0.043, p=0.94, df=1). When the confounding factor of age was considered, *Plasmodium* spp. intensity was negatively correlated with juvenile condition (χ^2=54.17, p=0.0001, df=1), but *Atoxoplasma* spp. intensity was positively correlated with juvenile condition (χ^2=5.39, p=0.02, df=1).

Isospora spp. prevalence (χ^2=2.98, p=0.08, df=1) nor intensity were correlated with condition in juveniles (χ^2=0.01, p=0.97, df=1). *Plasmodium* spp. prevalence (χ^2=0.112, p=0.91, df=1) and *Atoxoplasma* spp. prevalence (χ^2=0.04, p=0.82, df=1) were not correlated with body condition. When the effect of sex was controlled for, condition was positively correlated with infection in males (χ^2=45.70, p=0.00001, df=1), but prevalence was not significantly correlated with condition when sexes were controlled for (χ^2=1.07, p=0.3, df=1). *Atoxoplasma* spp. prevalence (χ^2=0.2, p=0.65, df=1), nor intensity (χ^2=1.65, p=0.19, df=1) were correlated with condition once the effect of sex of the bird was controlled for, and neither were *Isospora* spp. prevalence (χ^2=0.73, p=0.39, df=1), or intensity (χ^2=0.5, p=0.47, df=1).

4.4.6 Breeding status

Breeding birds had higher prevalence of *Plasmodium* spp. (χ^2=324.2, p=0.00001, df=1), and higher intensity (χ^2=161.17, p=0.00001, df=1). Breeding birds also had higher prevalence of *Atoxoplasma* spp. than non-breeders (χ^2=137.68, p=0.00001, df=1), but sample size for intensity was too small to provide meaningful results. There was not enough data to test for difference in *Isospora* spp. prevalence or intensity between breeding and non-breeding birds.

4.4.7 Moulting

Moulting birds had higher prevalence (χ^2=232.97, p=0.00001, df=1) and intensity (χ^2=162.89, p=0.00001, df=1) of *Plasmodium* spp. Moulting birds had higher *Atoxoplasma* spp. prevalence (χ^2=181.68, p=0.00001, df=1), but lower intensity (χ^2=77.53, p=0.00001, df=1). Moulting birds also had higher *Isospora* spp. prevalence (χ^2=51.53, p=0.0007, df=1), and higher intensity (χ^2=6.94, p=0.008, df=1). When the confounding factor with age was controlled for, moulting juveniles had higher prevalence (χ^2=10.97, p=0.0009, df=1) and intensity (χ^2=45.66, p=0.00001, df=1) of *Plasmodium* spp. Moulting juveniles also had higher prevalence (χ^2=20.0, p=0.00001, df=1) of *Atoxoplasma* spp. but lower intensity (χ^2=15.94, p=0.00001, df=1). There were not enough data to test *Isospora spp.* prevalence and intensity in juveniles only. When only adults were considered, prevalence was higher in non-moulting birds (χ^2=161.66, p=0.00001, df=1), but there was not enough data to reliably test for differences in moulting and non-moulting adults between the other parasites.

4. 5 DISCUSSION

4.5.1 Year

The current study did not find a difference in *Plasmodium* spp. prevalence across the three years, nor difference in intensity. A similar result was found for prevalence and intensity of *Atoxoplasma* spp. and *Isospora* spp., in line with a previous study on *Isospora* spp. prevalence in dark-eyed juncos over two years (Hudman *et al.*, 2000). Short-term studies on haematozoan have reported contrasting results on variation between years. In pied flycatchers, prevalence of *Haemoproteus* spp. did not vary across a two-year study in central Spain (Sanz *et al.*, 2001), nor between three breeding seasons in Norway (Dale *et al.*, 1996). *Plasmodium* spp. infection did not vary across years in a three-year study on blue tits (Cosgrove *et al.*, 2008), but Allander and Bennett (1994), found variation within a three-year study on prevalence and intensity of *Haemoproteus majoris* in a population of great tits in Sweden.

Prevalence of *Plasmodium* spp. depends on vector activity and availability of immunologically naïve hosts (Valkiunas, 2005). In the present study no data were available on mosquito abundance, therefore a definitive link between this vector and parasite prevalence cannot be made. Intensity of *Plasmodium* spp. infection depends on several factors, including the condition of the host (Atkinson and Van Riper, 1991) and the interaction between its immune system and the parasite (Wakelin and Apanius, 1997). Intensity did not vary across years, therefore factors affecting intensity of *Plasmodium* spp. in house sparrows at those sites probably had a similar effect on the birds across the three years.

The mode of transmission of *Atoxoplasma* spp. is presumed to be, like *Isospora* spp., through the faecal-oral route (Greiner, 2008). Factors affecting the exposure of hosts to these parasites did not vary across years, as indicated by lack of difference in prevalence. Intensity of *Atoxoplasma* spp. and *Isospora* spp. did not vary across years, suggesting that the number of oocysts ingested by the birds, and the ability of the latter to control infections, did not vary across years.

4.5.2 Seasons

A model suggests that prevalence of *Plasmodium* spp. should increase in late summer and autumn, when mainly juvenile birds born the same year provide susceptible hosts to the parasite, and it should decrease in winter when vector abundance decreases (Beaudoin *et al.*, 1971). In accordance with this theoretical approach, Applegate (1971) found that prevalence of *Plasmodium relictum* in experimentally-infected immunologically-naïve house sparrows peaked in March, April and May, followed by a rapid decline of prevalence from then until June, and a further decrease in prevalence in autumn, with a second, smaller peak, in December.

A study on blue tits showed two annual peaks of prevalence of *P. circumflexum* and *P. relictum* combined: one peak occurred in May/June, the other in September/October, and the lowest prevalence was recorded in December to February (Cosgrove *et al.*, 2008). Schrader and colleagues (Schrader *et al.*, 2003)

found peaks in prevalence of the *Plasmodium*-related genus *Haemoproteus* in July, and lowest prevalence in January and February in the red-bellied woodpecker.

In contrast with these theoretical and empirical results, in the current study prevalence of *Plasmodium* spp. was higher in winter than in any other season, but no further difference between seasons was detected. This result was unexpected, although a previous study on blackbirds had also found prevalence of *Plasmodium* to decrease from January to July (Hatchwell *et al.*, 2000). There are several possibilities for the unexpected result found in this study. First, prevalence might have really increased in winter. If mosquitoes had not hibernated due to mild winter temperatures, they could have continued to spread the infection through the winter months. This hypothesis seems unlikely, since there is no evidence that the past winters have been mild enough to prevent mosquito hibernation. Second, increased prevalence may be a sampling artefact: birds sampled were caught in gardens because they were attracted to feeders. Parasitised individuals could have been attracted to the feeder because the infection might have made them less able to find natural food. This hypothesis is also unlikely, because there is no evidence that feeders attract sub-optimal individuals, especially in winter. Third, higher prevalence in winter may have been the result of chronic infections becoming apparent. This seems the most plausible explanation, because very low-intensity chronic infections can remain undetected, especially during screening based on blood smears (Cosgrove *et al.*, 2008). However, Cosgrove and colleagues found that prevalence of *Plasmodium* spp. was absent in the blood of blue tits in November and December (Cosgrove *et al.*, 2008). Intensity did not vary between summer and autumn, it decreased in winter, and then further in spring, to increase again in summer. This is in accordance with a trade-off between breeding season effort and fighting of parasite infection in summer (Gustaffson *et al.*, 1994).

Atoxoplasma spp. and *Isospora* spp. also varied between seasons. *Isospora* spp. prevalence was higher in winter than in spring, and higher in winter than in summer. This result may be explained by a link between higher densities at bird feeders during the winter months (Chamberlain *et al*, 2005) and increased susceptibility to the parasite oocysts acquired through the faecal-oral route (Greiner, 2008), but no

data were available to test this hypothesis. Intensity of *Isospora* spp. was the highest in winter, it then decreased in spring and summer, and increased again in autumn. This pattern may reflect usage of bird feeders by garden birds, including house sparrows (Cowie and Hinsley, 1988), and since intensity of this parasite is dose dependent (Greiner, 2008), ingestion of a higher number of oocysts at bird feeders may increase intensity of infection, especially in winter and autumn when there are more susceptible juveniles in the population (Greiner, 2008). *Atoxoplasma* spp. prevalence did not show variation between seasons, and intensity was the highest in spring but dropped in summer, to increase again in autumn and winter. The link between ingestion of *Isospora* spp. oocysts and *Atoxoplasma* spp. is still unclear (Greiner, 2008), but the pattern seems to be consistent with a spring-relapse mechanism, closer to haematozoan that coccidian parasites, showing peaks in spring and then autumn.

4.5.3 Age

Birds of different age groups may vary in prevalence of infection if they are unequally exposed to that specific parasite (Wilson *et al.*, 2002). For *Plasmodium* spp., the difference in prevalence is thought to be due to difference in exposure to mosquito vectors, and adults in particular may have higher prevalence than juveniles because they have had more time to be exposed to infected mosquitoes (Wilson *et al.*, 2002). In this study, prevalence of *Plasmodium* spp. was higher in adults than in juveniles, in line with previous studies on *Plasmodium* spp. in blue tits (Cosgrove *et al.*, 2008; Wood *et al.*, 2007) and house sparrows (Valkiūnas *et al.*, 2006).

Migratory birds of the genus *Acrocephalus* (Waldenström *et al.*, 2002), and migratory shorebirds (Mendes *et al.*, 2005) also showed higher prevalence of *Plasmodium/Haemoproteus* spp. in adults. The same pattern was found for different species and parasites, for example *Leucocytozoon* spp. in adult great tits (Norris *et al.*, 1994), and *Haemoproteus* spp, in purple martins (Davidar and Morton, 1993), pied flycatchers (Sanz *et al.*, 2001b; Dale *et al.*, 1996) and great tits (Allander and Bennett, 1994). However, several authors have not found a difference in prevalence between ages. For example, in *Plasmodium* in native Hawaiian avifauna (Van Riper

et al., 1986), in *Haemoproteus* spp. in great tits (Allander, 1997) and in gray catbirds (Garvin *et al.*, 2003), in *Haemoproteus/Plasmodium* spp. infections in blackbirds (Evans *et al.*, 2009), and *Plasmodium* spp. in cirl bunting (Figuerola *et al.*, 1999).

There was no difference in prevalence of *Atoxoplasma* and *Isospora* spp. between age classes. This was in line with similar results on *Isospora* spp. prevalence in dark-eyed juncos (Hudman *et al.*, 2000), and in serins (*Serinus serinus*) (Lopez *et al.*, 2007), but it was in contrast with other studies which had found higher prevalence of *Isospora* spp. in young birds (Valkiūnas *et al.*, 2006).

Intensity of the infection may be due to the ability of the bird to control parasite proliferation once it has entered the host (Wakelin and Apanius, 1997), but the development of the infection depends on several factors, including the host-parasite interaction (Wilson *et al.*, 2002). In this study, *Plasmodium* spp. intensity was higher in juveniles than in adults, in line with other authors that found a similar result in native Hawaiian species (Van Riper *et al.*, 1986), and in *Haemoproteus* infections in great tits (Allander and Bennett, 1994), but mixed results for *Haemoproteus* and *Leucocytozoon* intensity in tawny owls (Appleby *et al.*, 1999), and no difference in intensity of Haemoparasites was found in house sparrows in temperate zone (Martin *et al.*, 2007). Juvenile feral pigeons (*Columba livia*) showed a decreased intensity of parasitaemia of *Haemoproteus* when re-caught as adults (Sol *et al.*, 2003). This may suggest that birds that survive the infection are able to control it, as suggested by experiment on *Plasmodium relictum* infection in Iiwi, which showed that chronically infected birds were able to withstand subsequent infections (Atkinson *et al.*, 1995). In an extreme case, Hõrak and co-workers (Hõrak *et al.*, 2001) found that adult great tits that had been positive for *Haemoproteus* spp. and cleared the infection survived better than uninfected adults, although the opposite was true for juveniles, in line with findings in the collared flycatcher (Nordling *et al.*, 1998).

There were no differences in intensity of *Atoxoplasma* or *Isospora* spp. between ages. This result was unexpected, since previous authors have suggested that these parasites and related genera are a problem mainly of young birds (Greiner, 2008),

and a study showed that intensity of *Isospora* spp. was higher in juvenile than in adult serins (Lopez *et al.*, 2007).

4.5.4 Sex

There are discrepancies in reported results of difference in prevalence and intensity of haemoparasites between males and females. In this study, males had lower prevalence and lower intensity of *Plasmodium* spp. than females. This result was in accordance with previous studies on the closely related *Haemoproteus* spp., which have found higher prevalence and intensity in female great tits (Ots *et al.* 1998). An analysis of 33 studies on haematozoan in birds showed that females tended to have higher prevalence of *Haemoproteus* spp. than males, but only during the breeding season, while *Plasmodium* spp. did not differ between sexes (McCurdy *et al.*, 1998). This may indicate that prevalence of parasite genera between sexes is due to several factors, and a general conclusion is difficult to attain without accounting for the ecology, the biological stage (e.g. breeding), and seasonality in the study considered. For example, a high number of studies focused on the breeding season. Norris and co-workers (Norris *et al.*, 1994) found that prevalence of *Leucocytozoon* was higher in females than males, but only birds in the breeding season were considered.

Applegate (1971) investigated prevalence of *Plasmodium relictum* in house sparrows and found higher prevalence in females, but only in April and May, and while he did not have enough data to analyse variation per month, he suggested that males may have higher prevalence at different times of the year. A study on blue tits found higher prevalence of *Plasmodium* spp. in males than females (Wood *et al.*, 2007).

In contrast with results in this study and those mentioned above, several authors have reported no difference in prevalence of different avian haematozoan between sexes of different species. There was no difference in *Plasmodium* spp. prevalence in blue tits between sexes (Cosgrove *et al.*, 2008), and a similar result was found for prevalence and intensity of *Plasmodium* spp. between male and female house sparrows in temperate regions (Martin *et al.*, 2007). *Haemoproteus* spp. prevalence

did not differ between sexes in cirl buntings (Figuerola *et al.*, 1999), in purple sand martins (Davidar and Morton, 1993), in house martins (Christe *et al.*, 2002), in pied flycatchers (Dale *et al.*, 1996), or in red-bellied woodpeckers (Schrader *et al.*, 2003). The prevalence and intensity of this parasite did not vary between males and females in great tits (Ots and Hõrak, 1998; Allander, 1997; Allander and Bennett, 1994), or in house sparrows (Gonzalez *et al.*, 1999), and no association between parasitism and sex was found for *Leucocytozoon* spp. in cirl buntings (Figuerola *et al.*, 1999). Some studies have reported no differences between the sexes, but they had considered prevalence of haematozoan without separating the genera (Evans *et al.*, 2009; Valkiūnas and Iezhova, 2001; Buchanan *et al.*, 1999; Allander and Bennett, 1995), which weakens the result, because hosts may vary in exposure and susceptibility to different parasite genera.

Atoxoplasma spp. and *Isospora* spp. followed an opposite trend between the two sexes for both prevalence and intensity. *Atoxoplasma* prevalence was higher in females, while *Isospora* spp. prevalence was higher in males; intensity of *Atoxoplasma* was higher in males, while intensity of *Isospora* spp. was higher in females. Previous studies on *Isospora* spp. have also found contrasting results: in blue-black grassquits females had higher prevalence and intensity than males (Aguilar *et al.*, 2008), but no difference in intensity of oocyst shedding was found between male and female blackbirds (Filipiak *et al.*, 2009).

4.5.5 Body condition

Parasitic infections are costly to their host (Goater and Holmes, 1997; Møller *et al.*,1990), not only because, by definition, parasites absorb energy from it (Clayton and Moore, 1997), but also because controlling the infection is energetically costly from the immunological point of view (Klasing *et al.*, 1991 cited in Wakelin and Apanius, 1997). Birds parasitised may, therefore, show decreased body condition as they use energy reserved to fight the infection.

In this study, birds infected with *Plasmodium* spp. did not show a different body condition to uninfected birds, in line with previous studies. In cirl buntings, no

difference in body weight was found between bird infected by *Plasmodium* spp. and uninfected birds (Figuerola *et al.*, 1999), and in Hawaii amakihi there was no difference in body mass between infected and uninfected breeding birds (Kilpatrick *et al.*, 2006). However, in this study intensity of the infection was negatively associated with body condition. This may be interpreted in two ways: *Plasmodium* had a negative effect on body condition, perhaps through direct use of the host's resources or, alternatively, birds in lower body condition did not have the energy to fight the infection, and *Plasmodium* replicated more in those weaker hosts.

These results may suggest that being infected did not relate with body condition, but in order to understand the relationship with intensity of infection one should conduct an experiment where body condition and parasitaemia were monitored in controlled environments [since loss of body mass may be due to several other factors, including breeding effort (Møller, 1997; Merilä and Wiggins, 1997) that could interact with parasite prevalence to influence intensity of the infection].

Previous experiments of this kind were conducted on birds, but they provided contrasting results. Captive Hawaii iiwi experimentally infected with *Plasmodium relictum* showed a difference response in body mass variation according to the dose of the *inoculum*: birds infected with high dose of the parasite showed reduced food consumption (provided *ad libitum*) and related loss of body weight than birds in low dose infection and control groups (Atkinson *et al.*, 1995). In another experiment on captive birds infected with *P. relictum,* Palinauskas and colleagues (Palinauskas *et al.*, 2008) found no effect of infection on body mass. Confounding factors on body condition may include the effort of breeding. Merilä and Wiggins (1997) manipulated blue tit broods to vary workload of the parents, and found that females with enlarged broods lost more weight as the breeding season progressed than females rearing control or reduced broods. This may be a confounding factor when associating body weight change to parasitaemia. In a medication experiment, Merino and co-workers (Merino *et al.*, 2000) found that female blue tits parasitised by *Haemoproteus majoris* had lower body mass at the end of the breeding season, unlike females that had been treated with primaquine to reduce infection. Navarro and co-workers found a correlation between diurnal fluctuation in body mass and

intensity of *Hameoproteus* spp. infection (Navarro *et al.*, 2003), but no difference in body mass between birds with or without *Haemoproteus* spp. (Navarro *et al.*, 2004). Body condition of male kestrels did not vary with parasite (*Haemoproteus* spp.) status, but during incubation parasitised females were in poorer condition than unparasitised ones (Dawson and Bortolotti, 2000). This may suggest a trade-off between energy allocated to breeding and to fighting infection, and birds in better body condition may have more resources to sustain both activities. Bennett and co-workers (Bennett *et al.*, 1988) found no correlation between body mass and *Haemoproteus* spp. prevalence nor intensity in migratory passerine birds. No effect of *Haemoproteus* spp. was found on the body mass of breeding females pied flycatchers (Sanz *et al.*, 2001). Male red-bellied woodpeckers parasitised by *Haemoproteus* spp. weighed less than non-parasitised birds, even when body mass was controlled for structural size (Schrader *et al.*, 2003). Body mass of temperate house sparrows was not affected by *Plasmodium* spp. intensity of infection (Martin *et al.*, 2007), and *Haemoproteus* spp. infection was not associated with body mass change in house martins (Christe *et al.*, 2002), nor in house sparrows (Gonzalez *et al.*, 1999).

There was no correlation between body mass and prevalence or intensity of *Atoxoplasma* spp. or *Isospora* spp.. These results are in contrast with previous studies on *Isospora* spp. that showed a negative effect of prevalence of the parasite on body mass in greenfinches (Horak *et al.*, 2004), and american goldfinches, although the latter species, the result was confounded by a decreased food-intake associated with the infection (McGraw and Hill, 2000). Intensity of *Isospora* spp. have also been associated with loss of body mass, for example in blue-black grassquits (Aguilar *et al.*, 2008; Costa and Macedo, 2005). An experiment on the effect of carotenoids on coccidian infection in house sparrows showed that birds infected with *Isospora* spp. lost more weight regardless of carotenoids supplementation in the first week post infection, but the effect disappeared after two weeks post-infection (Pap *et al*, 2009). However, in line with the current study, Baeta and colleagues (Beta *et al.*, 2008) conducted an experiment on the effect of carotenoids on *Isospora* spp., but they did not find an effect of *Isospora* spp. on body weight of blackbirds regardless of the carotenoid treatment. In pied flycatchers, an experiment in which females had been

removed to test for male parental care showed that males infected with *Haemoproteus* spp. did not lose more weight than uninfected males (Dale *et al.*, 1996).

4.5.6 Breeding

Breeding is a highly energetic activity, and a trade-off in birds between the ability to fight haematozoan infections and breeding effort has been suggested (Gustaffson *et al.*, 1994). This theory has been tested through observational studies (Sanz *et al.*, 2001; Allander and Bennett, 1995; Van Riper *et al.*, 1986), brood manipulation experiments (Knowles *et al.*, 2010b; Nordling *et al.*, 1998; Siikamäki *et al.*, 1997; Oppliger *et al.*, 1996; Richner *et al.*, 1995; Norris *et al.*, 1994), and medication experiments (Knowles *et al.*, 2010a; Marzal *et al.*, 2005). Several observational studies have shown a negative association between haematozoan prevalence and reproduction: Sanz and colleagues (Sanz *et al.*, 2001) found and association between prevalence of *Trypanosoma* spp. and desertion in pied flycatchers, and between *Haemoproteus* prevalence in females and hatching success in the same species, although fledging success and fledging mass was not affected. In an observational study on collared flycatcher, the authors did not find a negative effect between *Plasmodium* spp. prevalence in either or both rearing parents, and nestling performance (Szöllősi *et al.*, 2009).

Most of the above studies have, however, focused only on breeding birds. Very few studies attempted to compare parasite prevalence and intensity between breeding and non-breeding individuals. Those that did, compared birds during the breeding season with birds outside of the breeding season (McCurdy *et al.*, 1998), or it was unclear whether breeding and non-breeding birds had been compared in the same season (Van Riper *et al.*, 1986).

In this study, birds that were showing signs of active breeding were compared to non-breeding individuals, but the comparison was limited to the same time period for both typologies, so that they could be comparable without the confounding effect of seasonality. Plasmodium prevalence and intensity were higher in breeding birds than

in non-breeders, suggesting that there may be a trade-off between energy allocated to breeding and energy allocated to fighting *Plasmodium* spp. infection, in line with other authors (Knowles *et al.*, 2010a; Knowles *et al.*, 2010b; Knowles *et al.*, 2009; Marzal *et al.*, 2005; Merino *et al.*, 2000; Nordling *et al.*, 1998; Siikamäki *et al.*, 1997; Oppliger *et al.*, 1996; Ots and Hõrak, 1996; Richner *et al.*, 1995; Gustaffson, 1994; Norris *et al.*, 1994). In particular, a meta-analyses of experimental studies on birds on the trade-off between reproductive effort and blood parasitaemia showed that overall prevalence, and especially intensity, of parasite infection increased with reproductive effort (Knowles *et al.*, 2009). However, it cannot be excluded that differences in prevalence and intensity of *Plasmodium* spp. between breeding and non-breeding birds found in this study may be due to other factors, such as hormonal changes that have been shown to be implicated in the relapse of *Plasmodium* infection in spring in house sparrows (Applegate and Beaudoin, 1970). Results in this study are correlational, therefore reasons for differences in *Plasmodium* spp. prevalence and intensity between breeding and non-breeding birds can only be speculated. Furthermore, the small sample size of non-breeding birds may have weakened the result.

Contrary to this study, Van Riper and colleagues (Van Riper *et al.*, 1986) found no difference in intensity of *Plasmodium* spp. between breeding and non-breeding birds. However, although these authors looked for presence of brood patch and cloacal protuberance to ascertain reproductive status of the birds, they did not specify whether the non- breeding-bird sample had come from birds sampled in the same season, therefore other confounding factors may have influenced the intensity of parasitaemia. Siikamäki and colleagues (Siikamäki *et al.*, 1997) also failed to find any relationship between *Haemoproteus* spp. prevalence and fledging success, or weight, in pied flycatchers in natural, unmanipulated broods. However, when manipulated broods were considered, prevalence of the *Haemoproteus* spp. did not vary in males of enlarged or reduced broods, but intensity was lowest in males providing for reduced broods, and highest for males with enlarged broods, and the intensity was correlated with the number of nestlings (Siikamäki *et al.*, 1997).

McCurdy and co-workers (McCurdy *et al.*, 1998) conducted a meta-analyses of 33 studies, and found no difference in prevalence of haemoparasites in birds sampled during the breeding season or outside of it; however, no other effects were controlled for, nor it was specified whether birds during the breeding seasons were showing signs of breeding activity. Finally, an observational study on purple sandmartins infected with *Haemorporeus prognei* found no difference in clutch size between infected and uninfected females, but, unexpectedly, infected females fledged more young (Davidar and Morton, 1993).

Observing a relationship between reduced breeding success and high parasite prevalence or intensity does not help to elucidate what type of interaction the two variables have. Higher parasite levels may be responsible for reduced breeding success, or birds that are already of poor quality (and hence would have low breeding success) are also more susceptible to parasites. Experiments are the best way to investigate relationship between breeding and parasites, because several factors can be controlled for, and causal relationships can be potentially found. Unfortunately, it was not possible to conduct experiments during this study for three main reasons: first, reproductive data could not be collected because birds bred in natural nest holes under roof tiles for breeding; second, adults were very difficult to re-trap because they became aware of mist-nets after the first catching attempt, a problem found commonly by people working on this species (e.g. Martin II *et al.*, 2004); and finally, considering the two points above the only solution would have been to keep birds in captivity, which was beyond the scope of this study.

Several authors have taken the experimental approach. Oppliger and co-workers (Opplinger *et al.*, 1996) manipulated brood size of great tits by removing one egg in some broods, hence forcing the female to lay an extra-egg and increase her effort. They found an increased prevalence of *Plasmodium* spp. in enlarged broods than in control or reduced broods, supporting the hypothesis of a trade-off between breeding effort and parasite resistance. However, increased prevalence does not provide as much information on the relationship between breeding effort and resistance to parasites as intensity of infection would.

Mechanisms to avoid infections by *Plasmodium* spp. are still poorly understood, and they may include behavioural adjustments to avoid mosquito bites (Atkinson *et al.*, 1986), such as roosting with the bill and feet hidden in the feathers to avoid exposing bare parts (Atkinson *et al.*, 1986), or interactions between the parasite and the immune system of the host (Wakelin and Apanius, 1997). Furthermore, susceptibility to *Plasmodium* spp. seems to vary according to the species, and even to the single individual (Palinauskas *et al.*, 2008).

In a cross fostering experiments on great tits, Richner and co-workers (Richner *et al.*, 1995) found that males, but not females, of enlarged broods had higher prevalence of *Plasmodium* spp. than unmanipulated broods, and infected males had lower survival rate (calculated as return to the breeding season in the following year). This may suggest a trade-off between parasite defences and reproductive effort, and between current reproductive effort and parental survival (Charnov and Krebs, 1974). In an experiment on great tits, Ots and Hõrak (1996) reduced clutch size of birds, and found that intensity of *Haemoproteus* spp. infection was lower in females with reduced brood size. However, the authors had considered broods with unhatched eggs as reduced broods; this does not take into account that unhatched eggs may indicate quality of the female, and when those broods were excluded from the analyses the effect on parasite intensity was no longer significant. Knowles and colleagues (Knowles *et al.*, 2010b) conducted a brood-manipulation experiment in blue tits, and found that parents with enlarged broods had higher *Plasmodium* spp. intensity than unmanipulated parents, but prevalence did not vary. In collared flycatchers, females with enlarged broods had higher intensity of *Haemoproteus* spp infection, and sustained higher mortality than unparasitised females (Nordling *et al.*, 1998). Norris and colleagues (Norris *et al.*, 1994) found that male great tits with an enlarged brood had higher prevalence of *Leucocytozoon* spp., but no effect was found on females. The study, however, increased or reduced the brood size by 5 eggs, while remaining within the clutch size range of the species; a consequence was that large clutch size were generally reduced, and small clutch size generally enlarged (Sheldon and Verhurst, 1996).

Medication experiments can provide more compelling evidence for causal relationship between parasites and reduced breeding success. One such experiment used Malarone in blue tits to clear birds of *Plasmodium* spp. infection, and found increased hatching success and increased fledging success in treated females (Knowles *et al.*, 2010a). A similar experiment, conducted using Primaquine on house martins to control *Haemoproteus prognei* infection, showed that medicated parents had fewer parasites, larger clutch size, and higher hatching and fledging rates than untreated parents (Marzal *et al.*, 2005). Primaquine was also used on blue tits to reduce intensity of *Haemoproteus* spp. and prevalence of *Leucocytozoon* spp. infection (Merino *et al.* 2000). Nests of treated females had higher nestling survival and fledging than untreated females, supporting the theory of a trade-off between energy required for breeding and for controlling parasitic infections.

Finally, in this study *Atoxoplasma* spp. prevalence was higher in breeding birds compared to non-breeding birds, but sample size of intensity was too small to test for any difference, as was sample size on prevalence and intensity of *Isospora* spp.

4.5.7 Moulting

Moulting is an energy-demanding activity (Dawson *et al.*, 2000; Murphy and King, 1992), as suggested by experiments in which feather growth or quality was impaired by other concomitant energy-demanding activities (Hõrak *et al.*, 2004; Merilä and Wiggins, 1997). In this study, birds that were showing signs of moult had higher *Plasmodium* prevalence, and higher intensity, than non-moulting birds. Prevalence of *Atoxoplasma* spp. and *Isospora* spp. were also higher in moulting birds, which, however, had lower *Atoxoplasma* spp. but higher *Isospora* spp. intensities than non-moulting birds.

These results were partially due to differences between ages: moulting juveniles had higher prevalence than non-moulting juveniles, while moulting adults had lower prevalence. In young birds, this result may be the effect of longer exposure of moulting birds to vectors compared to non-moulting birds. Moulting juveniles are older than the non-moulting ones, because post-juvenile moult occurs about six weeks after fledging (Ginn and Melville, 1983), therefore they may have had more

time to become exposed to vectors, and parasites may have had more time to replicate, and hence to become apparent in the blood smear. Intensity in moulting juveniles was also higher than in non-moulting birds.

This result is difficult to interpret, since higher intensity may be due to a trade-off between the energy demand of moulting and the energy needed to control parasitic infection, but it may be due also to an effect of time or mortality of the host. *Plasmodium* spp. take between one to two weeks to reach a peak of parasitaemia in the host (Valkiunas, 2005; Bishop *et al.*, 1938), therefore younger birds may not have developed a level of parasitaemia that was high enough to be detected in the blood smear. A third explanation is linked to *Plasmodium* spp. pathogenicity, which can be very high (e.g. Valkiunas, 2005; Atkinson *et al.*, 1995), and younger birds may be more likely to die of the disease (Atkinson and Van Riper, 1991). This would result in older birds (being those that have survived) to have higher prevalence [because infection is never cleared completely (Bishop *et al.*, 1938)], and higher intensity than younger birds, because the intensity present in moulting birds effectively reflects the number of parasites that remain after the host has survived the peak of the parasitaemia. The parasites that were still apparent would have been the remnant of the infection that the bird had controlled enough to be still alive. In adult birds prevalence was higher in non-moulting birds; this effect could not have been due to the effect of concomitant breeding effort, because birds considered for moulting analyses did not show evidence of breeding within the moulting period. This result may suggest that birds that were not moulting were also not breeding, and hence might have been of lower quality. However, the very small sample size considered in the analyses weakens the interpretation of the results.

Higher prevalence of *Atoxoplasma* and *Isospora* spp. in moulting birds may also reflect increased exposure to the parasites, in particular if birds flock after the breeding season. Moulting birds had lower prevalence of the parasites, perhaps indicating that they were able to control the parasites more than non-moulting birds, which might have been lower quality individuals, because non-moulting house sparrows would have been younger birds in the case of juveniles, and non-breeders in the case of adults.

Studies on difference in parasitism between moulting and non-moulting wild birds are limited, and previous results on house sparrows have not found an effect of *Isospora* spp. on moulting parameters, such as speed of moulting (Pap *et al.*, 2009). However, that study was conducted on birds kept in captivity and experimentally infected with the parasite. Experimental studies using birds in captivity are essential to understand the causal relationship between variables, but those results cannot be applied to wild animals if conditions (such as food availability and rate of predation) are not kept realistically similar to those encountered in the wild.

4.6 CONCLUSIONS

This study showed that parasites varied between seasons, and with age, sex, condition, breeding status and moult of house sparrows, although a causal relationship between the variables was not possible due to the lack of an experimental approach.

There was no variation of either *Plasmodium*, *Atoxoplasma*, or *Isospora* spp. across years, suggesting that the parasites were stable in the population, or that the study period was not sufficiently long to identify patterns of variation in these pathogens, which depend on several factors including susceptibility of the hosts, their vector (Schall and Marghoob, 1995) and the ecology of the vectors (Wood *et al.*, 2007).

Previous authors (Cosgrove *et* al., 2008; Schrader *et al.*, 2003; Applegate, 1971; Beaudoin *et al.*, 1971) had identified a peak in haemoparasite prevalence in spring ('spring relapse'), when vectors appear after over-winter hibernation, and in autumn, when juveniles provide immunologically-naïve hosts. In contrast with these results, the current study found a peak in prevalence of *Plasmodium* spp. in winter, and the most plausible explanation is that a difficult season such as winter may make existing infections more apparent in the blood smears, although intensity was lower than in other seasons.

Theory predicts that adult birds may have higher prevalence of haemoparasites than juveniles, because they had more time to be exposed to vectors (Wilson *et al.*, 2002),

but they will have lower intensity of infection because their immune system is more capable of controlling the pathogen (Altizer *et al.*, 2006) as the surviving adults may have already been selected for. In accordance to this theory, this study showed that adults had higher prevalence of *Plasmodium* spp., but not of *Atoxoplasma* spp. or *Isospora* spp., than juveniles, but the latter had higher intensity of infection of *Plasmodium* spp., although there was no difference in intensity in *Atoxoplasma* or *Isospora* spp. infection between adults and juveniles.

Males have higher prevalence and intensity of parasitic infections in many taxa, due to their physiology and behaviour (Klein, 2004). This study found a sex-biased parasitism, but in the opposite direction to what the general theory predicts. Females had higher prevalence and intensity of *Plasmodium* spp. than males, but this result was in accordance with previous authors (Ots *et al.*, 1998; Norris *et al.*, 1994). Furthermore, females had higher prevalence of *Atoxoplasma* spp. but lower intensity than males, but the opposite was true for *Isospora* spp. infection. This result is difficult to explain. Differences in prevalence between the sexes should reflect different exposure to the parasite, but the suggested link between *Atoxoplasma* spp. and *Isospora* spp. (Barta *et al.*, 2005) should result in similar results for the two stages of the parasite between sexes. The scarce knowledge on the mechanisms behind the movement of *Isospora* spp. into the blood stream to become *Atoxoplasma* makes interpretation of these results difficult. It is only possible to speculate that the difference in prevalence and intensity of *Atoxoplasma* and *Isospora* spp. between males and females may be due to their different physiology.

Parasitic infections are costly to their host (Goater and Holmes, 1997), and birds parasitised may be in poorer body condition than unparasitised birds because they use their energy reserves to fight the infection. In this study only *Plasmodium* spp. intensity was significantly negatively correlated with body condition. This result may indicate that there is a trade-off between energy allocated to body condition and energy allocated to fighting and controlling the pathogen, or that individuals in low body condition are already of poor quality and not able to control the infection, but body condition and parasite intensity are not directly linked. Without an

experimental approach to control for the initial quality of the individual, it is impossible to tease these two factors apart.

Breeding and moulting are two high-energy demanding periods in the life-cycle of birds, and a trade-off between the ability to fight infections and these activities (Dawson *et* al, 2000; Gustaffson *et al.*, 1994). In this study prevalence and intensity of *Plasmodium* spp. were higher in birds showing signs of breeding, compared to birds that were not breeding within the same time period. This may suggest that there is a trade-off between the energy allocated to breeding and energy allocated to fighting pathogen, but a causal link can only be established through experiments using anti-malarial drugs. Birds that were showing signs of moult also had higher prevalence and intensity of *Plasmodium* spp. than birds that were not moulting, in the same period. This may be an indication of a trade-off between the energy needed for this activity, and controlling the infection, but the same size was not large enough to draw definite conclusions, and experiments that control the timing of moult may be useful to establish a causal link.

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Chapter 5

ASSOCIATIONS BETWEEN PARASITES, POPULATION TREND AND SUPPLEMENTARY FEEDING

5 . ASSOCIATIONS BETWEEN PARASITES, POPULATION TREND AND SUPPLEMENTARY FEEDING

5.1 INTRODUCTION

Diseases have the ability to control animal populations by modifying the host's chances of survival and/or fecundity and reproductive success (Anderson and May, 1979; May and Anderson, 1979). They can be defined as any departure from health that produces specific signs or symptoms (Pearsall, 1998), on a physiological, physical, reproductive or behavioural level (Friend *et al.*, 2001).

Diseases have been linked to population decline in several bird species. In North America, Hochachka and Dhondt (2000) showed a relationship between *Mycoplasma gallisepticum*, causing Mycoplasmal conjunctivitis, and the decline of the house finch (*Carpodacus mexicanus*) in eastern USA, and LaDeau and colleagues (LaDeau *et al.*, 2007) identified the outbreaks of the West Nile Virus as the cause of the decline of some North American bird species. Parasites, in particular, have been shown to have regulatory effect on some of their host populations. Hudson and colleagues (Hudson *et al.*, 1998; Hudson *et al.*, 1992b) found that the nematode *Thricostrongylus tenuis* had a regulatory effect on the Red Grouse population via decreased breeding success and winter survival. Van Riper and colleagues highlighted the role of *Plasmodium* spp. as regulatory agent in Hawaiian native bird species populations (Van Riper *et al.*, 1986). In the UK, the decline of the greenfinch (*Carduelis chloris*) has recently been shown to be a consequence of the protozoan *Trichomonas gallinae* (Robinson and Lawson *et al.*, 2010).

Parasites belonging to the genus *Plasmodium* are pathogenic in their avian hosts (Atkinson *et al.*, 2000; Atkinson *et al.*, 1995; Atkinson and Van Riper, 1991), but the evidence of epizootic die-offs caused by *Plasmodium* spp. parasites in the wild are scarce (Atkinson, 2008b), and the case of the aforementioned decline of Hawaiian

native bird species by *Plasmodium relictum* (Atkinson *et al.*,1995; Van Riper *et al.*, 1986) is the only example to date.

Diseases can regulate host population not only through reduced survival, but also through reduced reproduction (McCallum and Dobson, 1995). Field experiments have shown an association between prevalence and intensity of *Plasmodium* spp. and related haemosporidians, with reduced reproduction (Knowles *et al.*, 2010; Tomás *et al.*, 2007; Marzal *et al.*, 2005; Merino *et al.*, 2000). Knowles and colleagues (Knowles *et al.*, 2010) conducted a medication experiment on female blue tits using the anti-malarial drug Malarone, which cleared birds from erythrocytic-phase *Plasmodium* spp. chronic infections. Females treated with the anti-malarial drug had higher hatching and fledging success than control females, probably related to the higher provisioning rate than in controls. In another medication experiment on blue tits and *Haemoproteus* spp. infection, Tomás and colleagues (Tomás *et al.*, 2007) showed that females medicated with antimalarial drug Primaquine had lower intensity and higher provisioning rates than control birds, but the authors did not detect differences in fledging success or nestling condition.

Another medication experiment, this time on adult house martins (*Delichon urbica*), showed that birds treated with Primaquine had reduced intensity of *Haemoproteus prognei* infection (Marzal *et al.*, 2005). The authors found that medicated parents had lower intensity of the parasite, and their nest had larger clutch size, and hatching and fledging success than control groups, although they did not detect differences in fledging condition between the two treatments. Merino and colleagues (Merino *et al.*, 2000) also conducted a medication experiment on female blue tits using Primaquine to control intensity of *Haemoproteus majoris* and prevalence of *Leucocytozoon majoris*. The antimalarial drug reduced intensity of *H. majoris* and intensity of *L. majoris,* and nests of medicated females had higher fledging success than control groups, but no difference in condition of chicks was found between treatments.

Parasites of the genus *Isospora* are coccidian intestinal parasites closely related to *Eimeria* spp., which are known pathogens in commercial poultry (Greiner, 2008). *Isospora* spp. have the potential to cause the host's death if they destroy many epithelial cells of the intestine during the asexual reproduction, when multiple

merozoites which will infect new cells are produced (Greiner, 2008; Box, 1977). However, the pathogenicity of species of *Isospora* is considered low, and a problem affecting mainly young birds (Rossi *et al.,* 1997). In Poland, high prevalence of this parasite genus was associated with unhatched eggs and dead nestlings of tree sparrows and house sparrows (Kozłowski *et al*, 1991).

One of the main effects that intestinal *Isospora* spp. have on wild bird hosts is reduced nutrient uptake (Hõrak *et al.*, 2004). Reduced absorption of carotenoids, for example, may affect colouration of traits that are linked to this nutrient (Baeta *et al.*, 2008; Costa and Macedo, 2005; Hõrak *et al.*, 2004; McGraw and Hill, 2000). The effect of *Isospora* spp. on body mass of the host is unclear: some studies have found a reduced body mass associated with infection (Costa and Macedo, 2005; Kruszewicz, 1995), but others have failed to find such an association (Baeta *et al.*, 2008).

Parasites belonging to the genus *Atoxoplasma* are now considered extra-intestinal asexual phases of some species of *Isospora* (Greiner, 2008; Barta *et al.,* 2005) that are found in monocytes and lymphocytes in the blood (Campbell and Ellis, 2007). The diseases resulting from the parasites, atoxoplasmosis, is also referred to as "going-light" syndrome (Cooper *et al.*, 1989), because birds may stop eating and lose weight, look lethargic and have ruffled feather, as well as diarrhoea (Norton *et al.*, 1993). Atoxoplasmosis is usually considered a disease of young birds (Greiner, 2008) and asymptomatic in free-living wild birds, but in some cases stress may exacerbate the infection and pathology (Rossi *et al.*, 1997). Atoxoplasmosis has been identified as a major problem in the reintroduction captive programmes of the bali mynahs (*Leucopsar rothschildi*) (Partington *et al.*, 1989) caused by the host-specific *Isospora rothschildi* (Upton et al., 2001).

Food supply can limit the size of avian populations (Lack, 1954). Providing food for birds through supplementary feeding can enhance overwinter survival and breeding success. For example, Brittingham and Temple (1988) found increased overwinter survival in black-capped chickadees (*Poecile atricapillus*) that were provided with supplementary food over the winter months compared to control, unfed, birds. Improved overwinter survival was also found in willow tits (*Poecile montanus*) and

crested tits (*Lophophanes cristatus*) supplemented with seeds during winter (Jansson *et al.*, 1981). In house sparrows, food supplementation increased overwinter survival in rural environments (Hole *et al.*, 2002).

Supplementary feeding during winter can also have a positive "carry-over" effect into the breeding season. Robb and colleagues (Robb *et al.*, 2008) provided peanuts from November to the beginning of March in 5 woodland sites, and kept as many sites as control. They found that blue tits from supplemented woodlands laid earlier than conspecifics in control woodlands, and they had higher fledging success (but not larger clutch size or hatching success) than in control sites. In song sparrows (*Melospiza melodia*), supplementation of mealworms and seeds advanced laying date, and increased clutch size, nestling weight, number of fledglings, and nesting attempts compared to birds in unfed territories, but survival of fledged birds did not differ (Arcese and Smith, 1988). In contrast to this finding, a study on the effect of mealworm provisioning after hatching in yellow warblers (*Dendroica petechia*) showed no difference in survival of nestling (Lozano and Lemon, 1995).

House sparrow nestlings are fed preferentially on invertebrates by their parents, and vegetable matter increases in their diet until fledging (Summers-Smith, 1988; Seel, 1969; Summers-Smith, 1963). A study on house sparrows in Leicester highlighted a negative relationship between proportion of vegetable matter in the diet of nestlings and fledging success (Peach *et al.*, 2008; Vincent, 2005). Lack of invertebrates have been suggested as a limiting factor in the reproductive success of blue tits and great tits (Cowie and Hinsley, 1987), and of house sparrows (Seel, 1970) in urban and suburban environments.

A lack of invertebrates in the diet has therefore been put forward as a possible reason behind the decline of the house sparrow in urban environments (Peach *et al.*, 2008; Summers-Smith, 2007). The highest mortality of chicks in the Leicester study occurred in the first week after hatching (Peach *et al.*, 2008), and the authors suggested that this was linked to the lack of invertebrates in the diet, usually the predominant food in that initial period of life (Summers-Smith, 1963). However, other factors may act synergistically with lack of food to cause mortality of chicks, such as parasites.

In a study on causes of mortality in house sparrow nestlings, 50% of dead chicks age 1-5 days were positive for *Isospora lacazei*, while only 14% tested positive for coccidian parasites in the age group 6-10 days old, and 0% in dead nestlings over 10 days old (Kozłowski *et al*, 1991). Prevalence in ill-looking nestlings across the three age classes was 30%, 21% and 15% respectively, while apparently healthy nestling of all ages had about 5% prevalence of coccidian (Kozłowski *et al*, 1991). This may suggest that the effect of parasites can be present, but overlooked if these pathogens are not tested for. For example, reduced body mass can be an effect of food deprivation, but also parasites, which can actively consume nutrients from the host (Wobeser, 2008). In an experiment on *Isospora lacazei* in house sparrow and tree sparrow nestlings, Kruszewicz (1995) showed that this parasite caused loss in body mass of nestlings, and chicks that survived had suffered a reduction in body mass of 8.8% compared to non-infected birds.

Nutrients in the diet can also affect the resistance to infectious diseases through the immune system (Klasing, 1998). Proteins, in particular, are essential for cell-mediated responses, and lack of nutrients is associated with an impaired immune response (Chandra, 1977). Supplementary food may help by providing extra resources directly to the birds to fight infection, or by decreasing the workload of the parents feeding young. Wiehn and Korpimaki (1998) showed that female kestrels from supplemented nests had lower *Haemoproteus* spp. prevalence than females from non-supplemented nests. In Ural owls, Karell and colleagues (Karell *et al.*, 2007) found a similar result in female with *Leucocytozoon* spp. infection. In another raptor species, the tawny owl (*Strix aluco*), Appleby and colleagues (Appleby *et al.*, 1999) found a negative correlation between prevalence of *Haemoproteus* spp. and *Leucocytozoon* spp. and abundance of the owls' natural food, the field vole (*Microtus agrestris*).

In this study, half the sites were provided with mealworms during the breeding season (section 2.1.1), to test for the effect of food supplementation on parasite prevalence and intensity of birds in the colony. Nestlings fed on mealworms, which are rich in proteins (Bernard *et al.*, 1997), are expected to have lower prevalence and intensity of parasites.

5.2 AIMS

The aims of this chapter are to compare prevalence and intensity of *Plasmodium* spp., *Atoxoplasma* spp., and *Isospora* spp. in house sparrows between sites where the sparrow population is either:

a) Declining or non-declining.
b) Given supplementary food or not.

Interactions between trend and feeding status are also explored in case the effects of one variable depend on the status of the other. These associations are explored for both adult and juvenile sparrows.

5.3 METHODOLOGY

5.3.1 Description of the variables

Parasite prevalence and intensity

Parasite counts were obtained following the methodology outlined in chapter 2, sections 2.3.1 and 2.3.3. Parasite genera were analysed separately. Prevalence of infection is summarised as the percentage of birds infected, while intensity was calculated as the number of parasites per 10,000 erythrocyte for *Plasmodium* spp., the number of parasites per 100 leucocytes for *Atoxoplasma* spp., and as a score (from 0 to 5) of oocysists found in faecal samples for *Isospora* spp. (for more details see Chapter 2 section 2.3.3)

Sites

Eleven sites were successfully sampled for blood and faeces to test for parasitology (Table 5.1).

Population trends

Two surveys were carried out on each site every year, from 2005 to 2009, by the RSPB along fixed transect routes between March- April and then April-May, at one month interval, to count all male house sparrows, separating those that were chirping from those that were not chirping. Males are more conspicuous that females,

especially during the beginning of the breeding season when they are advertising their presence through chirping.

Analyses on changes in population size were performed using the highest count of chirping plus non-chirping males between the two visits. A measure of population trend for each site was calculated as the slope of the linear regression of the log male count against year.

Feeding regime: fed vs unfed sites

House sparrow nestling are fed mainly on an invertebrate diet by their parents (Summers-Smith, 1988). The RSPB conducted a large-scale experiment (described in section 2.1.1) to establish the role of lack of invertebrates in the diet of house sparrow juveniles in the decline of this species in London. The experiment involved the provision of 100g of live mealworm, twice a day, in 3 gardens per site during the breeding season (April to August) using special feeders (Fig. 2.2). Those sites are hereafter referred to as "fed sites". Gardens that were not provided with mealworms are hereafter referred to as "unfed sites". However, the unfed sites were not totally devoid of supplementary food, as garden owners usually provided seeds and/or peanuts. The amount of food provided to the birds by each house owner was not estimated, but all households roughly had the same number of feeders (usually 2), therefore this variable can be considered constant between and among fed and unfed sites.

5.3.2 Sample size

Please refer to table 5.3: results of prevalence per site provide, in brackets, the total number of birds sampled per each parasite, per site, and the number of infected birds used for analyses on intensity [i.e. (14/56 = "sample size used for intensity"/"total number sampled used for prevalence")].

5.3.3 Statistical analyses

Generalised linear models (glm) were applied using either parasite prevalence or intensity as the response variable, and population trend and feeding status as the explanatory variables. The rationale for this approach was to seek associations between parasitism and the two explanatory variables, without assuming a particular direction of causality. For these purposes, trend was transformed into a binary variable, classifying sites as either declining or non-declining. In the case of prevalence, the dependent variable was a matrix containing the numbers of infected and uninfected individuals at each site, and quasibinomial errors were specified to account for overdispersion. In the case of intensity, the dependent variable was the (log-transformed) mean number of parasites detected per individual at each site, with the gamma errors family specified, and weighting by the number of infected individuals found at each site.

As well as analysing all individuals together, the above tests were repeated using only adult or only juvenile birds. The aim of this was to test whether any associations between population trend or feeding regime and parasitism differed between adults and juveniles. Differences in prevalence and intensity in adults or juveniles can help to suggest possible mechanisms behind any patterns found (e.g. lower intensity in fed sites in juveniles may identify the effect of mealworm feeding on nestlings, while difference in adults may indicate a possible positive effect of feeding mealworms through reduction of workload of the parents).

The p-values reported in the results are those of the likelihood ratio test specifying the F distribution to control for overdispersion.

5.4 RESULTS

5.4.1 Site population trends and experimental feeding regime

Table 5.1. Site code, rate of population change (using log-transformed count of total males), trend category based on the rate of change, binary population trend , and experimental feeding regime. The significance of the regression slope for each site is in brackets.

Site code	Population change (ln(males))	Population trend	Fed/Unfed
31S	0.1418 (p=0.04)	Non-declining	Fed
73S	0.0068 (p=0.1)	Non-declining	Fed
51N	0.0256 (p=0.08)	Non-declining	Fed
86N	0.1374 (p=0.1)	Non-declining	Unfed
85N	-0.3088 (p=0.03)	Declining	Unfed
67N	0.1805 (p=0.07)	Non-declining	Fed
40S	-0.13513 (p=0.03)	Declining	Fed
33N	-0.2850 (p=0.02)	Declining	Unfed
59S	-0.04114 (p=0.05)	Declining	Fed
37S	-0.3222 (p=0.04)	Declining	Unfed
79S	-0.0519 (p=0.059)	Declining	Unfed

Table. 5.2. Summary of the results on the association between parasites and population trend and supplementary feeding. Plasm.= *Plasmodium* spp; Atox.= *Atoxoplasma* spp.; Isosp.= *Isospora* spp.; Prev.= Prevalence of parasites infection (calculated as infected/total); Int.=Intensity of infection (calculated as number of parasites detected per individual); Ad.= adult birds only; Juv. = juvenile birds only; df= degrees of freedom of the denominator.; N/A= not enough data available. Significant p-values are in bold.

			Trend			**Feeding status**		
Parasite	**Measure**	**Age**	**F**	**df**	**p**	**F**	**df**	**p**
Plasm.	Prev.	Both	2.37	10	0.15	0.159	10	0.69
Plasm.	Prev.	Ad.	3.43	9	0.09	0.02	10	0.88
Plasm.	Prev.	Juv.	0.025	8	0.99	1.20	8	0.3
Plasm.	Int.	Both	10.98	9	**0.01**	0.001	9	0.97
Plasm.	Int.	Ad.	6.32	9	**0.03**	0.0016	8	0.96
Plasm.	Int.	Juv.	17.43	7	**0.005**	0.02	6	0.89
Atox.	Prev.	Both	36.64	10	**0.0002**	14.02	10	0.004
Atox.	Prev.	Ad.	18.49	10	**0.0019**	2.13	10	0.17
Atox.	Prev.	Juv.	10.42	8	**0.014**	8.33	8	**0.023**
Atox.	Int.	Both	1.4	9	0.26	4.18	9	0.08
Atox.	Int.	Ad.	9.84	9	**0.013**	4.54	8	0.07
Atox.	Int.	Juv.	0.03	7	0.86	0.3	6	0.6
Isosp.	Prev.	Both	0.5	10	0.49	11.57	10	**0.007**
Isosp.	Prev.	Ad.	6.44	10	**0.031**	N/A	N/A	N/A
Isosp.	Prev.	Juv.	8.69	8	**0.02**	15.02	8	**0.006**
Isosp.	Int.	Both	0.22	9	0.68	N/A	N/A	N/A
Isosp.	Int.	Ad.	N/A	N/A	N/A	N/A	N/A	N/A
Isosp.	Int.	Juv.	0.22	2	0.71	N/A	N/A	N/A

Table 5. 3. Prevalence (infected/total) and mean intensity (calculated as number of parasites detected per individual) (standard error in brackets) of *Plasmodium* spp., *Atoxoplasma* spp and *Isospora* spp. per site. Site type: N= Non-declining, D= Declining, F= Fed, U= Unfed.

Site code (type)	*Plasmodium*		*Atoxoplasma*		*Isospora*	
	Prevalence	**Intensity**	**Prevalence**	**Intensity**	**Prevalence**	**Intensity**
31S (N/F)	0.25 (14/56)	10.78 (2.0)	0.37 (21/56)	2.2 (0.27)	0 (0/33)	N/A
73S (N/F)	0.22 (9/41)	34 (1.41)	0.36 (15/41)	4.4 (0.77)	0.29 (9/31)	3.4 (0.2)
51N (N/F)	0.18 (12/65)	19.41 (4.1)	0.46 (30/65)	2.8 (0.34)	0.06 (3/45)	3.3 (0.35)
86N (N/U)	0.25 (2/8)	17 (3.28)	0.37 (3/8)	1.7 (0.28)	0 (0/4)	N/A
85N (D/U)	0 (0/2)	N/A	0 (0/1)	N/A	0 (0/1)	N/A
67N (N/F)	0.18 (5/28)	21.3 (1.53)	0.39 (11/28)	2.1 (0.38)	0.12 (2/17)	2.0 (0.29)
40S (D/F)	0.26 (8/31)	4.5 (1.32)	0.16 (5/31)	1.4 (0.2)	0 (0/15)	N/A
33N (D/U)	0.43 (6/14)	40.3 (10.5)	0.2 (3/15)	1.0 (0)	0 (0/6)	N/A
59S (D/F)	0.5 (3/6)	99.6 (36.7)	0.5 (3/6)	1.0 (0)	0 (0/7)	N/A
37S (D/U)	0.1(13/101)	34 (8.63)	0.11 (11/97)	1.8 (0.2)	0.18(12/68)	3.2 (0.16)
79S (D/U)	0.39 (11/28)	72.7(14.6)	0.39 (11/28)	2.6 (0.83)	0 (0/15)	N/A

Table 5.4. Range of intensity of *Plasmodium* spp., *Atoxoplasma* spp and *Isospora* spp. per site. Site type: N= Non-declining, D= Declining, F= Fed, U= Unfed. Min= Minimum intensity; Max = Maximum intensity.

Site code (type)	*Plasmodium*		*Atoxoplasma*		*Isospora*	
	Min	**Max**	**Min**	**Max**	**Min**	**Max**
31S (N/F)	0	34	0	5	0	0
73S (N/F)	0	29	0	22	0	5
51N (N/F)	0	71	0	10	0	5
86N (N/U)	0	21	0	2	0	0
85N (D/U)	0	0	0	0	0	0
67N (N/F)	0	106	0	6	0	3
40S (D/F)	0	12	0	2	0	0
33N (D/U)	0	69	0	1	0	0
59S (D/F)	0	174	0	1	0	0
37S (D/U)	0	250	0	5	0	5
79S (D/U)	0	157	0	11	0	0

5.4.2 Declining vs non-declining

Plasmodium spp.

Prevalence of *Plasmodium* spp. infection was not significantly different between declining and non-declining sites even when adults and juveniles were considered separately. However, mean intensity of *Plasmodium* spp. was higher in declining than non-declining sites, and a similar result was found when the two age classes were considered separately.

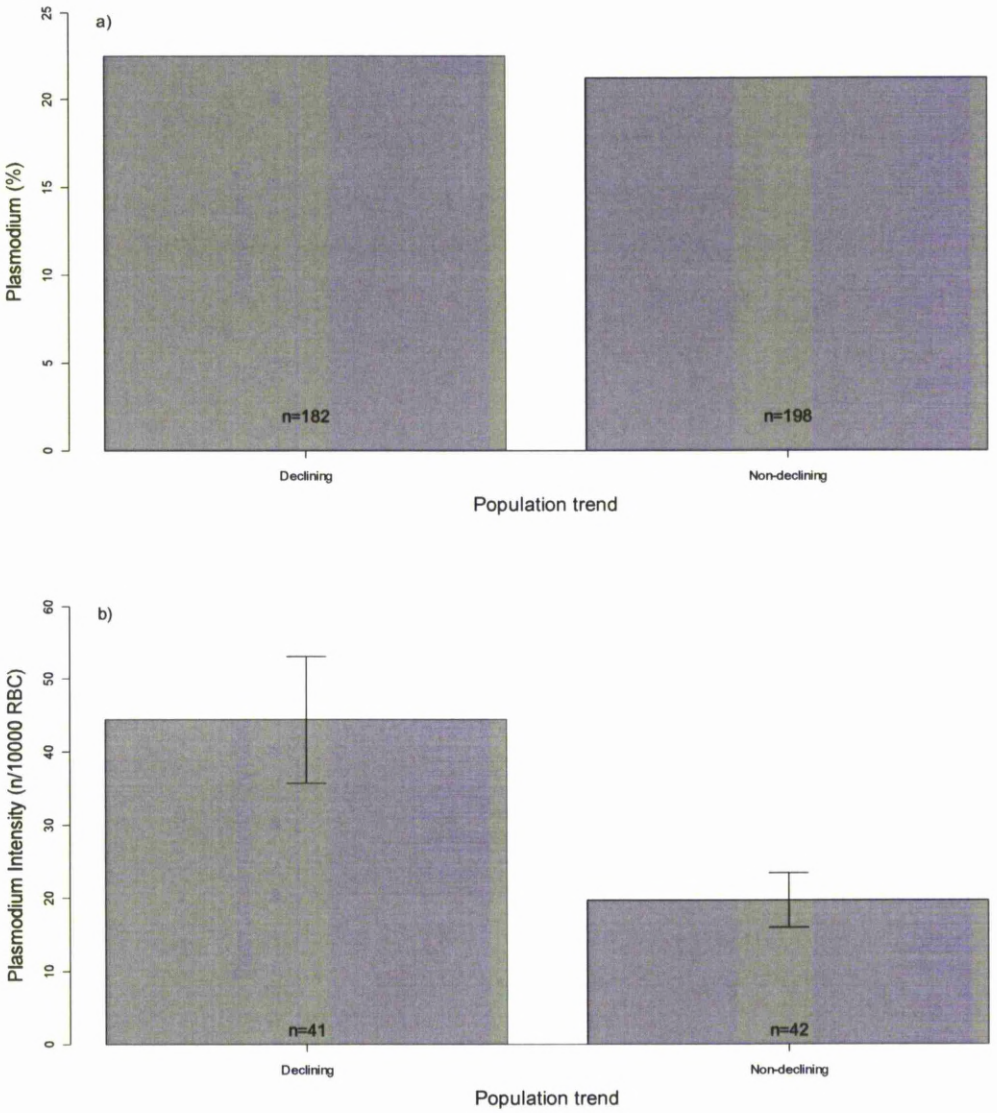

Fig. 5.1. *Plasmodium* spp. prevalence (a) and intensity (b) between sites with declining and non-declining population. Prevalence of *Plasmodium* spp. depends on whether birds encountered a vector of the parasite, and it is likely not to be related to the quality of the individual. Intensity of infection, on the contrary, depends on the ability of the individual to control the infection. In declining sites there may be, therefore, some characteristics of the birds beaten by the vector that make the individuals less able to control the infection.

Atoxoplasma spp.

Prevalence of *Atoxoplasma* spp. infection was significantly higher in non-declining sites, and a similar result applied when adults and juveniles were considered

separately. Only intensity of infection in adults was significantly different between population trends, with *Atoxoplasma* spp. intensity higher in declining sites.

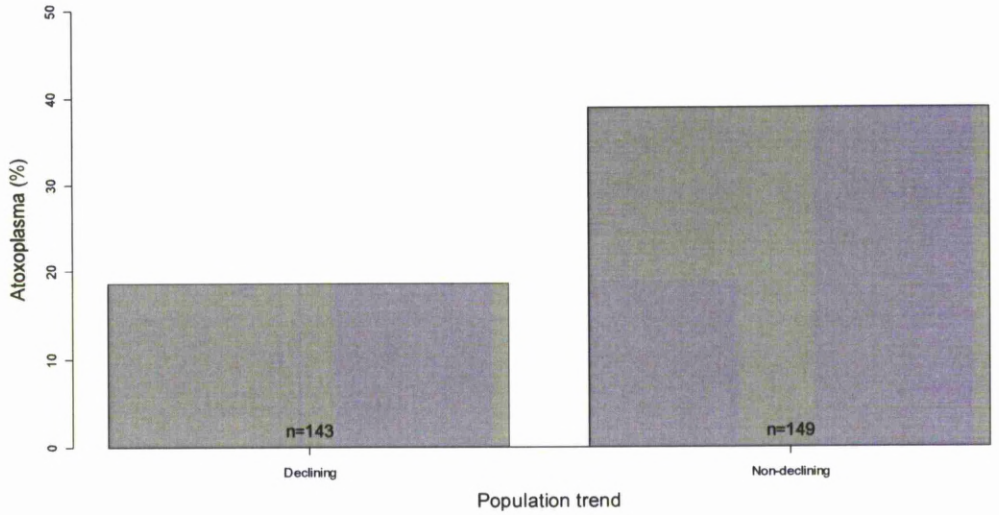

Fig. 5.2. *Atoxoplasma* spp. prevalence between sites with declining and non-declining population. A higher proportion of birds from sites of non-declining population carried *Atoxoplasma* spp. than individuals sampled at sites with declining population.

Isospora spp.

Prevalence of *Isospora* spp. infection was not significantly different between declining and non-declining sites However, prevalence was significantly different when the two age classes were considered separately, but the trends were in opposite directions. In adults, prevalence was higher in non-declining sites, but in juveniles prevalence was higher in declining sites. Mean intensity of *Isospora* spp. was not significantly different between declining and non-declining sites, even when the two age classes were considered separately.

5.4.3 Fed vs unfed sites

Plasmodium spp.

Prevalence and intensity of *Plasmodium* spp. infection were not significantly different between fed and unfed sites, even when adults and juveniles were analysed separately.

Atoxoplasma spp.

Prevalence of *Atoxoplasma* spp. infection was significantly higher in fed sites than in unfed sites, and the result remained significant when only juveniles were analysed. Mean intensity of *Atoxoplasma* spp. was almost significantly higher in unfed sites, but the result became strongly non-significant when juveniles were analysed separately.

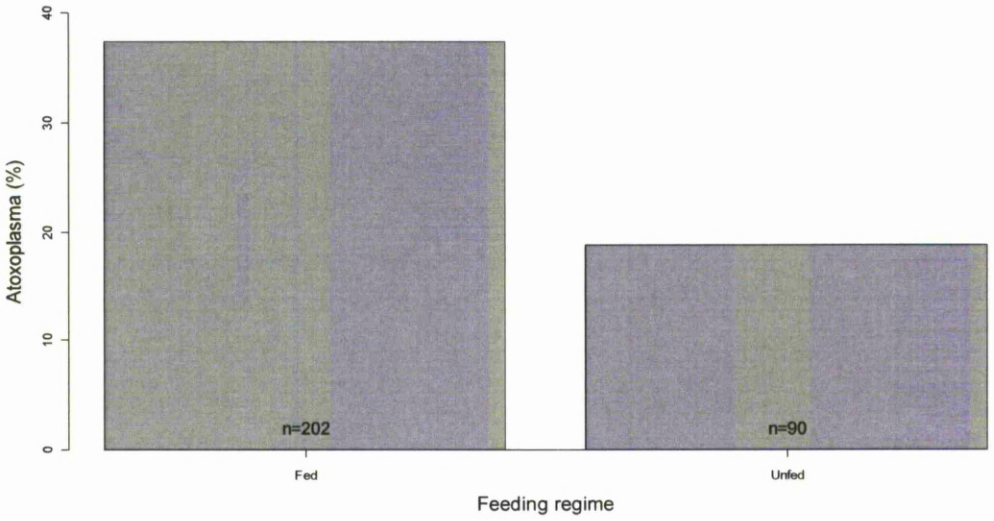

Fig. 5.3. *Atoxoplasma* spp. prevalence between sites in which mealworms had been provided or that were left as controls (unfed). Birds from fed sites may have been more likely to acquire the parasite because of congregation of individuals around a feeder in a short time window.

Isospora spp.

Prevalence of *Isospora* spp. infection was higher in fed sites than in unfed sites, even when juveniles where considered separately. It was not possible to compare intensity between fed and unfed sites because no birds were positive for *Isospora* spp. in unfed sites.

5.4.4 Interaction between population trend and experimental feeding regime

None of the interactions between population trend and feeding regime were significant, when any of the three parasites, and/or differences between age classes, were considered.

Table 5.5. GLM results for prevalence and intensity of parasites in relation to the interaction between population trend and feeding regime. Plasm.= *Plasmodium* spp; Atox.= *Atoxoplasma* spp.; Isosp.= *Isospora* spp.; Prev.= Prevalence of parasites infection (calculated as infected/total); Int.=Intensity of infection (calculated as number of parasites detected per individual); Ad.= adult birds only; Juv. = juvenile birds only; df= degrees of freedom of the denominator.; N/A= not enough data available.

Response term	**Age**	**F**	**Df**	**P**
Plasm. Prev.	Both	1.415	10	0.27
Plasm. Prev	Ad.	0.63	10	0.45
Plasm. Prev	Juv.	0.89	8	0.38
Plasm. Int.	Both	3.01	9	0.12
Plasm. Int.	Ad.	1.17	9	0.31
Plasm. Int.	Juv.	0.04	7	0.83
Atox. Prev.	Both	0.0031	10	0.95
Atox. Prev.	Ad.	0.67	10	0.43
Atox. Prev.	Juv.	0.08	8	0.78
Atox. Int.	Both	0.9	9	0.38
Atox. Int.	Ad.	0.41	9	0.54
Atox. Int.	Juv.	N/A	N/A	N/A
Isosp. Prev.	Both	3.75	10	0.10
Isosp. Prev.	Ad.	N/A	N/A	N/A
Isosp. Prev.	Juv.	0.71	8	0.43
Isosp. Int.	Both	N/A	N/A	N/A
Isosp. Int.	Ad.	N/A	N/A	N/A
Isosp. Int.	Juv.	N/A	N/A	N/A

5.5 DISCUSSION

Prevalence and intensity of infection can provide important insights into the possible dynamics of the host-parasite interaction (Tompkins *et al.*, 2002). Macroparasites can regulate their host population even when only a small proportion of individuals are infected (Tompkins *et al.*, 2002).

Prevalence of *Plasmodium* spp. was not different between declining and non-declining sites, but intensity of infection was higher in declining sites, even when adults and juveniles were considered separately. This suggests that *Plasmodium* spp. was present in the same proportion of hosts in declining and non-declining sites but that infected house sparrows in declining sites could not control the infection as well as birds in non-declining sites. The difference in intensity may, therefore, be due to some factors that make infected birds in declining sites less able to control the infection, and hence the parasites replicate more than in non-declining populations. Intensity of infection does not seem to be related to the number of sporozoites inoculated the first time (Palinauskas *et al.*, 2008; Permin and Juhl, 2002), but mortality may be higher in birds that are concomitantly bitten by several infected mosquitoes when the host is still uninfected (Atkinson *et al.*, 1995), although, based on results presented by Atkinson and co-workers (Atkinson *et al.*, 1995), such hosts do not seem to have a higher percentage of infected erythrocytes. Intensity does not increase when an individual with a chronic infection is bitten again by an infected vector (Atkinson *et al.*, 1995). Birds infected by *Plasmodium* spp. that do not succumb to the disease develop a degree of immunity to re-infection by the same species of *Plasmodium* for several years (Atkinson *et al.*, 2001), but they seem unable to clear the infection that remains chronic (Atkinson, 2008). If the host immune system is compromised by stress, or other factors including infection by other pathogens (Wright *et al.*, 2005), the intensity of infection may increase again ("relapse") (Atkinson and Van Riper, 1991).

Plasmodium spp. can cause high mortality in birds (Atkinson *et al.*, 2000; Atkinson *et al.*, 1995; Atkinson and Van Riper, 1991), and it has been suggested as cause of population declines in Hawaii endemic species (Van Riper *et al.*, 1986), and as factor limiting their recovery (Benning *et al.*, 2002; Atkinson *et al.*, 1995). Atkinson and

co-workers (Atkinson *et al.*, 2000) showed that in the immunologically-naïve Hawaii amakihi (*Hemignathus virens*) mortality from a single mosquito bite was 65% of infected birds, while mortality of iiwi that received a bite from 4-10 infected mosquitoes was 100% (Atkinson *et al.*, 1995).

The result of the current study suggests an association between *Plasmodium* spp. intensity and population decline of house sparrows, although the exact means of this interaction (increased mortality or reduced productivity) are not known. In this study, it was not possible to measure host fecundity and productivity, but medication experiments have shown that, even at low-intensity, chronic infection of *Plasmodium* spp. can affect breeding success, and hence ultimately fitness, of the host (Knowles *et al.*, 2010). However, correlational data in the Hawaiian amakihi found that nests with at least one parent with chronic *P. relictum* infection did not have lower breeding success than nests with both uninfected parents (Kilpatrick *et al.*, 2006). Data on productivity related to infection for the house sparrows in this study are needed to understand the effect that *Plasmodium* can have on the population dynamics. In addition to causing direct death and decreased breeding success, *Plasmodium* spp. can also negatively affect the behaviour of their host by exposing it to other causes of mortality, such as predation. Yorinks and Atkinson (2000) showed that juvenile apapane (*Himatione sanguinea*) experimentally infected with *Plasmodium relictum* went through a period of almost total inactivity, regardless of the outcome of the infection, which in the wild would have made them vulnerable to predators.

Proteins are essential for cell-mediated responses, and a lack of this nutrients in the diet is associated with an impaired cell-mediated immune response (Chandra, 1977). Mealworm are rich in proteins (Bernard *et al.*, 1997), therefore they should help nestling growth, and possibly help to fight infections. However, there was no apparent effect of mealworm supplementation on prevalence and intensity of *Plasmodium* spp. in this study. Lack of difference in prevalence was expected, because the mosquito vector should not be affected by supplementary mealworm food. Intensity of infection was expected to be lower in fed sites if the supplementation of food had given extra resources to young birds to control infections, or by decreasing the workload of parents foraging for their nestlings by

providing them with access to invertebrates. In order to test between these two possible effects of supplementary mealworm, the two age classes need to be analysed separately. However, when adults and juveniles were considered separately, no difference was found in prevalence or intensity between fed and unfed sites, suggesting a lack of effect of mealworm feeding on parasites in juveniles and adults. However, adults of fed and unfed sites should be compared only during the period when mealworms were provided (April to August) and until October, to test for any carry-over beneficial effects in the subsequent months. Unfortunately, there were not enough data to test the effect of adults specifically in the period April to October.

Results of this study are in contrast with previous findings. Saino and colleagues (Saino *et al.*, 1997) found that supplementation with protein-rich food in barn swallow nestlings was associated with higher PHA response than in non-supplemented nestlings, and the authors suggested that there was a link between food quality and ability to mount an immune response. The existence of a trade-off between resources allocated to fighting haematozoan infections and resources allocated to other traits has been suggested (Sheldon and Verhurst, 1996; Gustafsson *et al.*, 1994). Experiments have shown an association between increased breeding effort and increased parasite prevalence and intensity (meta-analyses in Knowles *et al.*, 2009). Supplementary food can decrease the workload of the parents feeding young, and hence reduce infections by haemoparasites. For example, Wiehn and Korpimaki (1998) showed that female kestrels from supplemented nests had lower *Haemoproteus* spp. prevalence than females from non-supplemented nests. In Ural owls, Karell and colleagues (Karell *et al.*, 2007) found a similar result in females with *Leucocytozoon* spp. infection. In another raptor species, the tawny owl (*Strix aluco*), Appleby and colleagues (Appleby *et al.*, 1999) found a negative correlation between prevalence of *Haemoproteus* spp. and *Leucocytozoon* spp. and abundance of the owls' natural food, the field vole (*Microtus agrestris*).

In this study, prevalence of *Atoxoplasma* spp. was higher in non-declining sites than in declining sites, even when adults and juveniles were considered separately. Overall intensity of infection did not vary, and a similar result was found when only juveniles were considered. However, adults had higher intensity in declining sites, suggesting that even if prevalence was higher in non-declining sites, birds in

declining sites either ingested a higher dose of the parasite, or they were less able to control the infection. The difference in prevalence indicates a difference in exposure. *Atoxoplasma* spp. originate from intestinal coccidiosis that infect leucocytes (Barta *et al.,* 2005), hence the parasite can be acquired from the faecal-oral route, for example by using bird-feeders. Prevalence of this parasite was higher in fed sites than in unfed sites, but intensity did not vary. Higher prevalence in fed sites may be due to supplementation of mealworms attracting more individuals to the feeder in a shorter time frame than normal bird food. In all sites birds were fed seeds by the garden owner, therefore contamination at bird feeders was always possible. However, garden owners reported an increased concentration of house sparrows around mealworm feeders soon after the food was provided, but the concentrated activity ceased when the mealworm feeder was empty, soon after it was filled (*pers. comm.*). Flocking of birds to the feeder could have increased chances of transmission of the parasite. Interestingly, when age classes were considered separately, only juveniles had higher prevalence in fed sites, while in adults the difference was not significant. This may be explained if juveniles used mealworm feeders after fledging, and became in contact with infected faeces. However, there were no data to test this hypothesis, and link between *Atoxoplasma* spp. infection and *Isospora* spp. presence is still unclear (Greiner, 2008). The interaction between feeding regime and population trend was not significant, indicating that the two factors, in the case of *Atoxoplasma*, did not have a synergistic effect. Intensity of infection did not vary overall with population trend, feeding regime, nor it was explained by their interaction. The pathogenicity level of *Atoxoplasma* spp. is still unclear, but it seems to be higher in young individuals and those under great stress (Rossi *et al.*, 1997). The results of this study do not suggest an association between population trend and this parasite, but they showed an association between its prevalence and mealworm feeding, possibly due to increased flocking around mealworm feeders.

Overall prevalence of *Isospora* spp. did not differ between declining and non-declining sites, but lack of significance may have been a consequence of opposite results in adults and juveniles. In adults prevalence was higher in non-declining sites, while juveniles had higher prevalence in declining sites. Prevalence reflects exposure to the parasite, and in declining sites juveniles might have been more exposed to

Isospora spp. oocysts than in non-declining sites, but the opposite was true for adults.

Reasons for difference in exposure can only be speculated, but limited food resources, for example, in declining sites might have caused young birds to visit a smaller number of feeding sites where they might have ingested infective oocysts. A link between feeding and prevalence of *Isospora* spp. was found in sites with mealworm supplementation, in which prevalence of this protozoa was higher both overall and in juveniles, but not enough data were available to test for diffences in adults. A similar explanation to the difference in prevalence in *Atoxoplasma* spp. can be suggested here: *Isospora* spp. oocysts are transmitted through the faecal-oral route, and aggregation of birds in a short time period is conducive of transmission between several individuals (Greiner, 2008). Time between visits of individuals to feeders may be important in the transmission of this parasite, which has been shown to be prone to desiccation and UV damage (Martineau *et al.*, 2009). However, *Isospora* spp. have to sporulate in the environment before becoming infective (Greiner, 2008). Sporulation can occur after 12-18hrs depending on temperature and humidity conditions (Langkjær and Roepstorff, 2008), therefore faeces shed by birds during the second (afternoon) mealworm feeding aliquot would have had time to sporulate overnight and be infective in the morning when the first aliquot of mealworm of the day was provided. The intensity of *Isospora* spp. infection did not differ between declining and non-declining sites, but there were no birds positive in unfed sites to compare between feeding regimes, nor to test the interaction between feeding regime and population trend.

Pathogenicity of *Isospora* spp. oocysts in the wild is considered low, but young birds seem to be more prone to develop the infection into a disease. For example, a higher prevalence of *Isospora* spp. oocysts were found in faeces of nestling tree sparrows and house sparrows that were ill or dead compared to apparently healthy birds of the same age (Kozłowski *et al*, 1991). Previous infection by a species of *Isospora* does not seem to confer resistance to re-infection by the same species of parasite (Hõrak *et al.*, 2004), hence re-infection can occur.

Results from this study do not show a link between presence of *Isospora* spp. parasites and population decline, but higher prevalence was found in juveniles in declining sites. Feeding of mealworms may increase aggregation of birds to an area, facilitating transmission of the parasite.

Avian populations fluctuate naturally, but often irregularly, across time due to several factors, and usually many years of surveying are needed to understand population trend (Newton, 1998). Population trend in this study was based on changes (or absence thereof) in population numbers over 5 years. It is possible that this length of time was not sufficient to record the true direction of population trend of the sites considered, because fluctuations within the long-term trend can occur, and the five-year period considered for this study may reflect one of this fluctuations. However, parasites can also fluctuate between years and seasons (Wilson *et al.*, 2002), and the aim of this study was to detect an association, not a causal link, between declining or non-declining status of a site, and presence and intensity of the parasite considered. Furthermore, overall decline of house sparrows at a national level is now reaching a plateau, and the main decline occurred before 1993 (Robinson *et al.*, 2005). Inference on a causal link, or association before this study started, are not possible without long-term data on parasitology and its interaction with other ecological factors such as reproduction and survival.

5.6 CONCLUSIONS

There was an association between *Plasmodium* spp. and population trend. Prevalence of *Plasmodium* did not differ between declining and non-declining sites, but intensity was higher in declining site, and the same result was obtained when adults and juveniles were considered separately. This may suggest that, while the proportion of birds infected in the two sites was not different, infected birds in declining site may be less capable of fighting the infection, and have higher intensity of *Plasmodium* spp. as a result. *Plasmodium* spp. prevalence and intensity did not differ between fed and unfed sites, and the interaction between feeding regime and population trend was not significant overall, nor when adults and juveniles were considered separately.
Prevalence of *Atoxoplasma* spp. was unexpectedly higher in non-declining sites, even when age classes were considered separately. Intensity of *Atoxoplasma* spp.

was significant only in adults, which had higher parasitemia in declining sites. This may suggest that, whilst a higher proportion of adults were infected in non-declining sites, those infected in declining sites had higher intensity, either because they were infected with a higher count of oocysts or because they were less able to control the infection, which proliferated. *Atoxoplasma* spp. prevalence was higher in fed sites, as expected from a parasite that is transmitted through the faecal-oral route and is more likely to be transmitted in crowded assembly of birds that flock to food sources, in this case the mealworms. In juveniles, prevalence of *Isospora* spp. was higher in declining sites, while adults from non-declining sites had higher prevalence. Prevalence of *Isospora* spp. was higher in fed site, and a similar explanation suggested for *Atoxoplasma* spp. is plausible.

Plasmodium is the most pathogenic of the three parasite genera found in this study, and its intensity was higher in declining sites. This result is correlational, hence it is not possible to ascertain whether the relation was causal. In order to identify a causal relationship between *Plasmodium* spp. and the decline of the affected population, a long-term experiment should be performed, in which all house sparrows in a population were experimentally infected with *Plasmodium* spp.. The population could then be monitored to test whether all individuals perished, whether they could successfully reproduce, or the colony was supplemented by new incoming birds. Such an experiment would obviously be characterised by practical and ethical problems, hence a correlational study is a good, albeit not perfect, alternative.

Plasmodium spp. can affect the host through mortality, but also reduced breeding success, and they can predispose their host to predation by affecting its behaviour. Without data on productivity of single birds, or at site level, it is not possible to fully understand what impact this parasite can have on the host population level, but it is likely to play a synergistic role, with other unidentified factors, in the trend of the house sparrow populations considered in this study.

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Chapter 6

ASSOCIATIONS BETWEEN PARASITE INFECTION AND HOUSE SPARROW SURVIVAL

6. ASSOCIATIONS BETWEEN PARASITE INFECTION AND HOUSE SPARROW SURVIVAL

6.1 INTRODUCTION

Theory predicts that parasites are costly to their hosts (Goater and Holmes, 1997; Møller *et al.*,1990), affecting their survival and fitness, depending on the pathogenicity of parasite and the complex interaction with the host's immune system (Wakelin and Apanius, 1997). Malarial parasites belonging to the genus *Plasmodium* can be highly pathogenic (Atkinson and Van Riper, 1991), and can cause high mortality in their avian host (Atkinson, *et al.* 2000; Yorinks and Atkinson, 2000; Atkinson *et al.*, 1995; Van Riper *et al.*, 1986). Pathogenicity of *Atoxoplasma* spp. is still focus of debate (Greiner, 2008), but mortality due to this parasite has been reported in different species of birds, including black siskins (Rossi *et al.*, 1997) and in captive bali mynahs (Partington *et al.*, 1989).

The impact of haemoprotozoan on adult survival has been addressed in experimental studies (Atkinson, *et al.* 2000; Yorinks and Atkinson, 2000; Atkinson *et al.*, 1995; Van Riper *et al.*, 1986) but studies proving their effect on free-ranging wild birds are scarce (Marzal *et al.*, 2009). Prevalence of *Haemoproteus* spp. was associated with lower return rate of migratory passerines (Nordling *et al.*, 1998) and raptors (Dawson and Bortolotti, 2000), and *Plasmodium* spp. prevalence was associated with lower return to the breeding site of sedentary species (Richner *et al.*, 1995). However, confounding factors such as condition-mediated survival could not be excluded (Martinez-de-la Puente *et al.*, 2010). Sub-optimal individuals may have lower body condition, and they may incur a higher cost of infection by haematozoan, which results in lower survival (Hanssen *et al.*, 2003).

A further confounding factor in estimating survival due to parasite is age: changes in first-year survival can account for the magnitude of population change (Robinson *et* al., 2004; Peach *et al.*, 1999). Juveniles, in general, have higher parasite prevalence and or intensity than adults (Dawson and Bortolotti, 1999; Allander and Bennett, 1994), and their survival is often lower (Sol *et al.*, 2003; Horak *et al.*, 2001). Infected

adults have already been selected for resistance to the parasite, and they may have acquired immunity to it (Sol *et al.*, 2003; Van Riper *et al.*, 1986). Survival analyses considering parasites should also consider sex-biased survival. Sex of the host is important in determining intensity of the infection (Klein, 2004; Møller *et al.*, 1998), although differences between sexes depend on several factors (Klein, 2004).

There is a trade-off between resources allocated to present and future efforts (Williams, 1966). One such trade-off seems to be between reproductive effort and parasite resistance and control of infection (Knowles *et al.*, 2010; Nordling *et al.*, 1998, Allander, 1997; Richner *et al.*, 1995; Norris *et al.*, 1994), therefore haematozoan may mediate the survival costs of reproduction. Correlational studies have failed to show a strong effect of haematozoan on survival of breeding birds (Stjernman *et al.*, 2004), but a medication experiment found higher survival of medicated females (but not males) to the following breeding season (Martinez-de la Puente *et al.*, 2010).

Parasites may also decrease survival of their host by altering their behaviour and making them more prone to other mortality agents. Helminths may increase vulnerability of birds to predators (Hudson *et al.*, 1992), and prevalence of haematozoan has been associated with risk of predation (Møller and Nielsen, 2007), possibly by affecting the behaviour of the host (Yorinks and Atikinson, 2000), although stress of predation could also cause higher parasite intensity through immunosuppression (Navarro *et al.*, 2004).

Winter is usually the time when a bird is most likely to die (Perrins, 1974), and bird population levels are usually at its lowest towards the end of the cold season (Newton, 1998). Prolonged periods of cold weather can affect survival (Robinson *et al.*, 2007; Peach *et al.*, 1995). Over-winter survival has traditionally been linked to food availability (Lack, 1954), day length, and weather (Perrins, 1974), but several factors may interact, and weather can influence survival indirectly, by affecting bird habitat or food supply, or directly if birds die of hypothermia (Newton, 1998). Reduced food availability may weaken the immune system (Chandra, 1997), and hence it may predispose the individual to direct or indirect mortality due to pathogens (Newton, 1988).

6.2 AIMS

The aims of this chapter are:

1. To investigate over-winter survival of house sparrows in relation to *Plasmodium* spp. prevalence and intensity of infection.
2. To investigate over-winter survival of house sparrows in relation to *Atoxoplasma* spp. prevalence and intensity of infection.

6.3 METHODOLOGY

6.3.1 Description of the variables

Parasite prevalence and intensity

Parasite counts were obtained following the methodology outlined in chapter 2, sections 2.3.1 and 2.3.3. Only the genus *Plasmodium* and *Atoxoplasma* have been considered due to small sample size for *Isospora.* Parasite genera were considered separately. Prevalence of infection indicated the percentage of birds infected, while intensity indicated the level of the infection of parasitised individuals.

Survival

All birds were fitted with a metal ring (see chapter 2 section 2.2.1) and a unique combination of three colour rings to identify the individual from afar. One colour ring was fitted above the metal one, and two on the other leg.

Each site was surveyed by the RSPB, using fixed transects, once a month from September to February to record presence and identity of colour-ringed birds. In this study, survival was divided between three groups within that period: autumn (September and October), early winter (November and December), and late winter (January and February) to represent differences in potential challenge that the birds may face from the weather. Autumn corresponds to the post-juvenile dispersal period (Fleischer *et al.*, 1984; Summers-Smith, 1963), the weather is still largely mild, with temperatures not dropping below freezing. Early winter corresponds to a progressive deterioration of the weather, with temperatures dropping, especially at night. Late winter corresponds to months of potentially inclement weather, low temperatures, and highest mortality in small passerines (Newton, 1998).

Age

Age of house sparrows was ascertained using plumage description described by Svensson (Svensson, 1992). For further details on the problem of ageing house sparrow, please see section 2.2.1. In analyses between the ages that do not differentiate between sexes, the data had come from pooled sample of both sexes.

Sex

Sexing of birds was done on plumage (Svensson, 1992). Juveniles are monomorphic and resemble adult females until they begin the post-juvenile moult. For details on sexing house sparrows please see section 2.2.1. In analyses between the sexes that do not differentiate between ages, the data had come from a pooled sample of both ages.

Condition

Condition was calculated as the residuals of the regression of body mass against tarsus length, to control for the structural size the birds.

6.3.2 Sample size

Table 6.1. Sample size for analyses of parasite prevalence and intensity in autumn, and for analyses of the interactions with age, sex, and condition. Prev= Prevalence; Int.= Intensity. (Number overall sampled/ Number infected). Ad= Adult; Juv=Juvenile; Cond= body condition.

	Overall	**Ad**	**Juv**	**Male**	**Female**	**Cond**
Plasm. Prev	163/ 65	58/ 41	105/ 24	61/ 33	45/ 25	58/ 41
Plasm. Int.	22/ 23	10/ 11	12/ 2	6/ 7	10/ 5	10/ 11
Atox Prev.	160/64	57/ 41	103/ 23	61/ 32	44/ 25	160/ 64
Atox Int.	51/ 16	16/ 11	35/ 5	19/ 5	13/ 10	51/ 16

Table 6.2. Sample size for analyses of parasite prevalence and intensity in winter, and for analyses of the interactions with age, sex, and condition. Prev= Prevalence; Int.= Intensity. (Number overall sampled/ Number infected). Ad= Adult; Juv=Juvenile; Cond= body condition.

	Overall	**Ad**	**Juv**	**Male**	**Female**	**Cond**
Plasm. Prev	186/ 49	76/ 30	10/ 19	74/ 20	54/ 21	186/ 49
Plasm. Int.	23/ 13	11/ 11	12/ 2	8/ 5	10/ 6	23/ 13
Atox Prev.	183/ 48	75/ 30	108/ 18	74/ 21	53/ 21	183/ 48
Atox Int.	54/ 14	19/ 9	35/ 5	20/ 4	16/ 8	54/ 14

Table 6.3. Sample size for analyses of parasite prevalence (Prev.) and intensity (Int.) in late winter and interactions with age, sex, and condition. (Number overall sampled/ Number infected). Ad= Adult; Juv=Juvenile; Cond= body condition.

	Overall	**Ad**	**Juv**	**Male**	**Female**	**Cond**
Plasm. Prev	191/ 45	78/ 29	113/ 16	8/ 19	55/ 20	191/ 45
Plasm. Int.	28/ 8	15/ 7	13/ 1	10/ 3	12/ 4	28/ 8
Atox Prev.	188/ 44	77/ 29	111/ 15	78/ 18	54/ 20	188/ 44
Atox Int.	58/ 10	22/ 6	36/ 4	21/ 3	18/ 6	58/ 10

6.3.3 Statistical analyses

Re-sightings (binary variable 0-1) from the three time groups were used separately as response variable, and parasite prevalence or intensity as explanatory variables. Interaction between parasite and age or sex of the host was also tested. Samples coming from the same sampling unit cannot be considered independent (Crawley, 2007), therefore birds sampled from the same sites are pseudoreplicated. In order to account for this, Mixed Effect Models were applied using the level of pseudoreplication (in this case "site") as random effect, and specifying the error family as quasibinomial because the binary response was overdispersed.

The significance of each explanatory variable was calculated by comparing the model with the variable to a model without the variable using the likelihood ratio test, with maximum likelihood, as suggested by Douglas Bates (2006). The p-values reported in the results are those of the likelihood ratio test specifying the F distribution to control for overdispersion (Crawley, 1993). All analyses were conducted in R 2.11.1 (R Development Core team, 2010) using Tinn-R R (Development Core team, 2010) as text interface.

6.4 RESULTS

6.4.1 Autumn

Re-sighting in September/October was higher in adults than in juveniles (χ^2=7.99, p=0.004, df=1), but there was no difference between re-sighting when sex (χ^2=0.13, p=0.71, df=1) or condition (χ^2=0.25, p=0.61, df=1) were considered.

Birds re-sighted in September/October had higher prevalence of *Plasmodium* spp. (χ^2=49.82, p=1.6e^{-12}, df=1) but lower prevalence of *Atoxoplasma* spp. (χ^2=54.46, p=1.5^{e-13}, df=1) than birds that were not re-sighted. Interaction between *Plasmodium* spp. prevalence and age was not significant in explaining re-sighting probability (χ^2=1.13, p=0.28, df=1). Interaction between *Atoxoplasma* spp. prevalence and age was also not significant (χ^2=0.54, p=0.45, df=1). Interaction between parasite prevalence and sex of the host was not significant when *Plasmodium* spp. were

considered (χ^2=1.83, p=0.39, df=1), nor when *Atoxoplasma* spp. were considered (χ^2=3.30, p=0.19, df=1). Interaction between parasite prevalence and condition was not significant when *Plasmodium* spp. infection was considered (χ^2=0.56, p=0.45, df=1), nor when *Atoxoplasma* spp. were considered (χ^2=0.25, p=0.61, df=1).

Re-sighted birds did not vary in intensity of *Plasmodium* spp. infection (χ^2=1.18, p=0.27, df=1), nor *Atoxoplasma* spp. (χ^2=0.5, p=0.47, df=1) intensity to non-re-sighted individuals. Interaction between intensity and age was not significant for either *Plasmodium* spp.(χ^2=2.4, p=0.12, df=1) or *Atoxoplasma* spp. (χ^2=0.9, p=0.34, df=1). Interaction between parasite intensity and sex of the host was not significant when *Plasmodium* spp. were considered (χ^2=0.55, p=0.45, df=1), nor when *Atoxoplasma* spp. were considered (χ^2=0.023, p=0.96, df=1). Interaction between parasite intensity and condition of the host was not significant when *Plasmodium* spp. were considered (χ^2=0.09, p=0.97, df=1), nor when *Atoxoplasma* spp. were considered (χ^2=3.62, p=0.056, df=1), although the latter was almost significant.

6.4.2 Early winter

Re-sighting in November/December did not vary with age (χ^2=2.42, p=0.11, df=1), with sex (χ^2=0.22, p=0.63, df=1), or condition (χ^2=0.09, p=0.76, df=1).

Birds re-sighted in November/December had higher prevalence of *Plasmodium* spp. (χ^2=34.36, p=4.5e-9, df=1) but lower prevalence of *Atoxoplasma* spp. (χ^2=34.12, p=5.16e-9, df=1) than birds that were not re-sighted. Interaction between *Plasmodium* spp. prevalence and age was not significant in explaining variation in re-sighting (χ^2=2.09, p=0.14, df=1), as was interaction between *Atoxoplasma* spp. and age (χ^2=0.41, p=0.51, df=1). Interaction between parasite prevalence and sex of the host was not significant when *Plasmodium* spp. were considered (χ^2=0.35, p=0.83, df=1), nor when *Atoxoplasma* spp. were considered (χ^2=1.17, p=0.42, df=1). Interaction between parasite prevalence and condition was not significant when *Plasmodium* spp. infection was considered (χ^2=1.21, p=0.27, df=1), nor when *Atoxoplasma* spp. were considered (χ^2=0.16, p=0.68, df=1).

Non-resighted birds had higher intensity of *Plasmodium* spp. infection than re-sighted individuals, but the difference was not significant at the canonical 5% level (χ^2=2.93, p=0.08, df=1), while there was no difference in re-sighting associated with *Atoxoplasma* spp. intensity of infection (χ^2=0.03, p=0.84, df=1). Interaction between intensity and age was almost significant (χ^2=2.94, p=0.08, df=1), but interaction between intensity of *Atoxoplasma* spp. and age was not significant (χ^2=1.71, p=0.19, df=1). Interaction between parasite intensity and sex of the host was not significant when *Plasmodium* spp. were considered (χ^2=0.83, p=0.36, df=1), nor when *Atoxoplasma* spp. were considered (χ^2=1.66, p=0.19, df=1). Interaction between parasite intensity and condition of the host was not significant when *Plasmodium* spp. were considered (χ^2=0.35, p=0.55, df=1), nor when *Atoxoplasma* spp. were considered (χ^2=0.08, p=0.77, df=1).

6.4.3 Late winter

Re-sighting in January/February did not vary with age at the conventional 5% level (χ^2=3.57, p=0.058, df=1), or with sex (χ^2=0.76, p=0.38, df=1), or condition (χ^2=0.07, p=0.79, df=1) were considered.

Birds re-sighted in January/February had higher prevalence of *Plasmodium* spp. than birds that were not re-sighted (χ^2=39.20, p=3.8e-10, df=1) but lower prevalence of *Atoxoplasma* spp. (χ^2=45.11, p=1.85 e-11, df=1). Interaction between *Plasmodium* spp. prevalence and age was not significant in explaining variation in re-sighting (χ^2=0.73, p=0.38, df=1). Interaction between *Atoxoplasma* spp. prevalence and age was also not significant (χ^2=0.03, p=0.84, df=1). Interaction between parasite prevalence and sex of the host was not significant when *Plasmodium* spp. were considered (χ^2=1.17, p=0.55, df=2), nor when *Atoxoplasma* spp. were considered (χ^2=1.88, p=0.38, df=2). Interaction between parasite prevalence and condition was not significant when *Plasmodium* spp. infection was considered (χ^2=0.30, p=0.51, df=1), nor when *Atoxoplasma* spp. were considered (χ^2=0.51, p=0.47, df=1).

Non-re-sighted birds had higher *Plasmodium* spp. intensity than re-sighted individuals (χ^2=7.16, p=0.007, df=1), even when only birds that were re-sighted in

the first period were considered (χ^2=14.54, p=0.0001, df=1). There was no association between *Atoxoplasma* spp. intensity and re-sighting probability (χ^2=0.016, p=0.89, df=1). Interaction between intensity of *Plasmodium* spp. and age was not significant (χ^2=2.05, p=0.14, df=1), and neither was interaction between *Atoxoplasma* spp. intensity and age (χ^2=0.44, p=0.5, df=1). Interaction between parasite intensity and sex of the host was not significant when *Plasmodium* spp. were considered (χ^2=2.42, p=0.11, df=1), nor when *Atoxoplasma* spp. were considered (χ^2=1.11, p=0.29, df=1). Interaction between parasite intensity and condition of the host was not significant when *Plasmodium* spp. were considered (χ^2=0.42, p=0.51, df=1), nor when *Atoxoplasma* spp. were considered (χ^2=3.53, p=0.06, df=1), although the latter was close to the canonical 5% significance.

6.5 DISCUSSION

6.5.1 Autumn

In the first period, defined as "autumn", birds that were re-sighted had higher prevalence of *Plasmodium* spp. than non-re-sighted birds. This result may seem counter-intuitive, but it is in line with other studies that have found higher survival of infected adult great tits (Hõrak *et al.*, 2001). This pattern may be due to the high virulence of *Plasmodium* spp. infection in immunologically-naïve hosts (Atkinson and Van Riper, 1991), which causes high mortality in young birds, which hence leave the population. In the period September/October adults were re-sighted more than juveniles, probably because most young birds will have left the colony by then, either through death or dispersal, which occurs at this time of the year (Fleischer *et al.*, 1984; Summers-Smith, 1963).

Adult birds showed in general higher prevalence of *Plasmodium* spp. infection than juveniles but lower intensity (Chapter 4), suggesting that adults can control the level of infection, probably due to a developed immunity (Atkinson *et al.*, 1995). This would provide an explanation for higher re-sightings of infected individuals. In this first period there also was no difference in intensity of *Plasmodium* spp. infection between re-sighted and non-re-sighted individuals, possibly because levels of

infection are still kept under control by the birds during this period of relatively unchallenging weather conditions.

Interaction between *Plasmodium* spp. prevalence or intensity and age, sex, and condition were not significant, suggesting that those variables did not interact to affect re-sighting probability. Sex and condition alone also did not explain resighting probability. In some taxa males tend to have a shorter-life span than females, due to differences in physiology (e.g. testosterone levels lower immune responses) and behaviour (Klein, 2004).

Birds in lower body condition may also be expected to survive less, as they may have less energy reserves to withstand periods of food scarcity (associated with bad weather, for example) (Newton, 1998), or to fight infections (Wobeser, 2008). Unfortunately, direct evidence of condition-mediated survival in birds is lacking, because of the difficulty of separating the confounding effect of initial low quality of the individual.

These results are surprising, because previous authors have suggested that survival differed between sexes, and that sex, condition, and age interacted with haematozoan infection to affect survival. A study on *Plasmodium* spp. prevalence in great tits showed that males, but not females, with enlarged brood had higher prevalence than males from control or reduced broods (Richner *et al.*, 1995), indicating a sex-biased in prevalence of parasite due to different level of parental provisioning rate. Survival of great tits was also not related to prevalence of *Haemoproteus* spp., age, or sex separately, but infection status was related to survival in an age-dependent manner (Hõrak *et al.*, 2001). Survival of females in collared flycatchers was also correlated with *Haemoproteus* spp. intensity of infection (Nordling *et al.*, 1998). However, in that study, survival was measured as failure to return to the breeding ground in the subsequent two years, which is a long time frame in which several factors apart from parasite infection could have affected return rates, including food shortage in the wintering ground (Newton, 2008), especially since survival rate in the study was lower in first-year females. In feral pigeons, survival of birds infected by *Haemoproteus* spp. was lower in juveniles than in adults, and it depended on intensity of infection, but not on body condition (Sol *et al.*, 2003). Furthermore,

surviving juveniles still had higher intensity than surviving adults, but intensity decreased in re-captured birds as they progressed with age, suggesting acquired immunity (Sol *et al.*, 2003). These studies have focused on breeding bird and overwinter survival. However, in the first time group considered here, September/October (=autumn), the effect of winter was not yet applicable, therefore lack of significance of the interaction between condition and parasites on survival was expected, but lack of effect of the interaction between *Plasmodium* spp. and sex or age was surprising.

Atoxoplasma spp. prevalence was lower in re-sighted birds than in non-resighted birds, although intensity did not vary. This could be due either to higher mortality associated with this parasites, especially in young birds (Greiner, 2008; Rossi *et al.*, 1997), which is supported by the adult-biased number of re-sighted individuals, or an artefact of re-sighted individuals to adults, which are reported to have lower prevalence and intensity of the parasite (Greiner, 2008; Rossi *et al.*, 1997). However, the link between age and *Atoxoplasma* spp. susceptibility has not been demonstrated, and the interaction between parasite prevalence (or intensity) and age were not significant, hence the age-dependent explanation is not supported in this case.

Interactions between *Atoxoplasma* spp. and sex or condition were also not significant. This result was surprising, because a significant interaction between the parasite and condition were expected, since the disease is also known as "going-light syndrome " (Cooper *et al.*, 1989); however, this result suggests that difference in re-sighting associated with *Atoxoplasma* spp. is not mediated by condition. This is in contrast with a medication experiment on intestinal parasite-condition mediated survival in Eider ducks (Hanssen *et al.*, 2003) which suggested that survival of sub-optimal females may be due to a synergistic effect of low body condition and cost of parasitism. They provided female Eiders with the anti-helminths Panacur and found that survival to the following year was higher for treated females of low quality, compared to low-quality untreated females, but not effect was found for good-quality females. However, in that study they did not identify parasite species, nor quantify helminth parasitism before or after treatment, therefore it is not possible to infer pathogenicity of parasites involved, nor establish the extent to which the anti-helmith has contributed to the result.

6.5.2 Early winter and late winter

Re-sighting in early or late winter did not vary with age, sex, or condition.
Re-sighted birds had higher prevalence of *Plasmodium* spp. than non-re-sighted birds both in early and late winter. This result may seem counter-intuitive, but it is in line with other studies that have found higher survival of infected adult great tits (Hõrak *et al.*, 2001).

This pattern may be due to the high virulence of *Plasmodium* spp. infection (Atkinson and Van Riper, 1991), which causes high mortality in young birds, which hence leave the population. The surviving individuals are those that have controlled the infection, mainly adults but some juveniles as well, and the parasite enters a chronic state (Hõrak *et al.*, 2001), hence the higher prevalence in surviving birds. However, probability of re-sightings changed with intensity of *Plasmodium* spp. infection as winter progressed.

In autumn there was no difference in intensity between birds that had been re-sighted and those that had not, but the difference was statistically significant at the end of winter, with birds that had higher intensity of *Plasmodium* spp. less likely to be seen. This can be interpreted primarily in two ways: birds with higher intensity of infection died of the disease, or the disease made the birds less obvious and hence underrepresented in the survey. *Plasmodium* spp. are highly pathogenic (Atkinson and van Riper, 1991), and mortality has been shown to be correlated with intensity of the infection (Atkinson *et al.*, 1995).

It is possible that birds with higher infection had succumbed to the disease, or the disease had predisposed them to other causes of mortality, for example predation (Møller and Nielsen, 2007; Yorinks and Atkinson, 2000), and hence were not re-sighted. However, birds with high infection level might have been affected by the disease, which affects the behaviour of the host (lethargy, inactivity, etc. (Yorinks and Atkinson, 2000)), therefore those birds might have been less conspicuous and hence underrepresented in the survey. None of the interactions between *Plasmodium* spp. and sex, age or condition were significant, indicating that the relationship between re-sighting probability and intensity of infection was not confounded by any

of these variables. This result is unexpected, because juveniles have, in general, higher prevalence of haematozoan infection than adults (e.g. Sol *et al.*, 2003; Dawson and Bortolotti, 1999; Allander and Bennett, 1994), as is also indicated in this study (see Chapter 4), and juveniles tend to suffer lower survival than adults (Lack, 1954), hence a parasite-age mediated lower survival was expected, as found by previous authors (Sol *et al.*, 2003).

One confounding factor in the analyses was that at the end of winter non-resighted individuals might have been those dispersed by autumn. In order to control for dispersal outside the survey area, only birds that were re-sighted in September/October were included, hence including birds that had not dispersed. By that time of the year, post-juvenile dispersal should be completed (Fleischer *et al.*, 1984; Summers-Smith, 1963), hence birds still seen in the area should be those that are staying for the winter. The negative relationship between intensity of *Plasmodium* spp. and re-sighting probability at the end of winter was still significant when only those birds were considered, providing some support of the theory that non-resighted birds have died rather than dispersed, especially since each period comprised two months, and the probability of re-sighting was higher than if only one month had been considered, because lack of re-sighting might have been due to chance.

Re-sighted birds had lower prevalence of *Atoxoplasma* spp. than non-resighted individuals in early and late winter, although intensity was not significantly different. An effect of this pathogen seems to be loss of body weight (Cooper *et al.*, 1989), although the interactions between *Atoxoplasma* spp. and body condition were not significant in explaining re-sighting probability, therefore body condition does not seem to mediate parasitism and survival. Intensity of *Atoxoplasma* spp. was not associated with probability of being re-sighted, not even when age was controlled for. This result may reflect two aspects: first, pathogenicity of this parasite is considered to be generally low (Greiner, 2008; Rossi *et al,* 1997), although it can be a problem in young birds (Greiner, 2008) and birds in captivity (for example, it has been associated with mortality in captive bali mynahs (Partington *et al.*, 1989)); second, intensity of *Atoxoplasma* spp. in this study was low (Table 4.2), therefore it was unlikely to correlated with survival probability.

Sex of the host was not a significant factor in prevalence or intensity of either parasite associated with re-sighting probability. This result was unexpected, not only because these parasites showed differences in prevalence and intensity between sexes (results in chapter 4, sections 4.4.3 and 4.4.4), but also because a sex bias in survival probability has been reported in several species (Klein, 2004). This result may indicate that, if non-resighting is equalled to death, survival in the population considered is not sex biased. There may be other behavioural differences between the sexes that make them more or less easy to spot and hence record.

6.6 CONCLUSIONS

Intensity of *Plasmodium* spp. infection was negatively correlated with the probability of an individual to be re-sighted at the end of winter, even when only birds re-sighted in the first period were included. This indicates that the parasite is linked to the undetectability of the individual, and that birds have not been lost due to dispersal, or they would have been absent in the first period September/October, too. *Plasmodium* spp. may have reduced survival of the individual, or they may have affected the bird's behaviour and made it less obvious and hence underrepresented in the survey.

Survival analyses have several limitations, including biases in the re-sighting of the individuals, and underlying assumptions such as that lack of re-sightings is identified with death of the individual rather than with its dispersal. The sedentary nature of house sparrows is well known, and after post-juvenile dispersal they tend to not move colony (Summers-Smith, 1988).

Survival analyses could also be carried out using other statistical programmes, like MARK, which allow one to model differences in detectability and survival for each age class and for each co-hort. In this study all birds considered were sampled in the same calendar year than the re-sighting took place, but differences in time lapsed between sampling and re-sighting was not controlled for. This might have biased some of the results, because birds that had already survived several months may have an inherited higher survival probability (because they would have already been selected for) than birds sampled close to the re-sighting event.

Another limitation is implicit in the sampling of haemoparasites, because a one-off blood sample provides only a snapshot of the intensity of parasitaemia in that specific moment (Wobeser, 2008), but inference of the development of the infection is not possible outside an experimental approach.

Even considering all the biases in the analyses, the reduction in survival in late winter months probably reflects a true association.

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Chapter 7

GENERAL DISCUSSION

7. GENERAL DISCUSSION

7.1. INTRODUCTION

The house sparrow is a small passerine bird belonging to the genus *Passer* (Sibley and Monroe, 1990). It used to be a ubiquitous bird in habitats associated with human beings, in urban, suburban and agricultural settings. The species was once so common and widespread that it was considered a pest in the 19th and 20th centuries, and eradication programmes were introduced (Summers-Smith, 1963). Rural populations of this species started to decline in the UK in the 1970s, and a decade later the species started to decline in urban and suburban areas, too (Robinson *et al.*, 2005).

The decline of the urban house sparrow is not evenly distributed throughout the British Isles: the decline has been marked in London, Edinburgh, Dublin, Bristol, Norwich, and Glasgow (Sanderson, 1996; Dott and Brown, 2002; Prowse, 2002; Bland, 1998; Paston, 2000; Summers-Smith, 1999), but other cities, such as Manchester and some urban populations in Wales and South West England have not reported a negative population trend (Robinson *et al.*, 2005). The London house sparrow population declined by 68% during the period 1994-2009 (Risely *et al.*, 2010), although the peak decline in urban environments elsewhere in Great Britain occurred in the period 1983-1994 (Robinson *et al.*, 2005).

Hypotheses for the decline in farmland populations include changes in agricultural practice and reduced food availability (Chamberlain *et al.*, 1999; Robinson and Sutherland, 2002; Hole *et al.*, 2002). Several hypotheses have been suggested for the decline of the urban house sparrow, including lack of nest sites (Noble and Eaton, 2002), increased predation pressure from cats (*Felis catus*) (Beckerman *et al.*, 2007; Baker *et al.*, 2005), and sparrowhawks (*Accipiter nisus*) (Bell *et al.*, 2010; MacLeod *et al.*, 2006), along with increased competition by sympatric species, such as pigeons (*Columba livia* and *Columba palumbus*) (Bland, 1998), the advent of lead-free petrol in the 1990s that led to the release of harmful substances (Summers-Smith, 2007), mobile phone masts (Balmori, 2009; Balmori and Hallberg, 2007), and food scarcity by reducing survival of

nestlings (Peach *et al.*, 2008). None of these hypotheses have been able to undisputably explain the decline of the house sparrow.

A remaining hypothesis for the decline of the house sparrow in Great Britain, and one which has not yet been tested, is that this is due to infectious disease. It is this hypothesis that I test in this thesis.

Disease can be defined as any departure from health that produces specific signs or symptoms (Pearsall, 1998), on a physiological, physical, reproductive or behavioural level (Friend *et al.*, 2001), and pathogens are organisms (bacteria, virus, fungi and parasites) that are capable of causing disease (Pearsall, 1998). Parasites are organisms that live in or on a host, from which they derive nutrients (Clayton and Moore, 1997), and they can be pathogens if they have the potential to cause disease (Clayton and Moore, 1997).

The cost of parasitism is not always obvious. Apparently neutral effects of parasites may be due to (Wobeser, 2008): a) an effectively trivial cost to the host resulting in a not-detectible effect, b) the cost is present but tolerable, specifically in those individuals that have enough energy to afford losing resources to the parasites without incurring significant negative effects on other functions (but this equilibrium might be altered with changing environmental circumstances); c) the cost of parasitism is masked by other factors, such as predation; d) the cost of parasitism is substantial but the individuals are examined/sampled at the wrong time of the year, or at the wrong life stage, or the cost of parasitism could be paid later in life.

The effect of parasites on population dynamics is, therefore, very difficult to investigate. However, some studies have shown a link between parasites and the population dynamics of their hosts. For example, Hudson *et al.* (1992b) found that the nematode *Thricostrongylus tenuis* had a regulatory effect on a red grouse population via decreased breeding success and winter survival. Van Riper *et al.* (1986) highlighted the role of *Plasmodium* spp. as a regulatory agent of populations of Hawaiian native birds. Bunbury

and colleagues found that *Trichomonas gallinae* and *Leucocytozoon marchouxi* were associated with decreased survival of the endangered Mauritian pink pigeon (*Columba majeri*) (Bunbury *et al.*, 2008; Bunbury *et al.*, 2007). On the North American continent, Hochachka and Dhondt (2000) showed a causal relationship between *Mycoplasma gallisepticum* infection, causing Mycoplasmal conjunctivitis, and the decline of the house finch (*Carpodacus mexicanus*) in eastern USA, and LaDeau *et al.* (2007) identified West Nile Virus as a cause of decline of some North American bird species. In the UK, the decline of the greenfinch (*Carduelis chloris*) has recently been shown to be a consequence of infection with the protozoan *Trichomonas gallinae* (Robinson and Lawson *et al.*, 2010).

Wild birds are natural reservoirs of bacteria, protozoa, helminth parasites and viruses. Garden birds are at particular risk of contracting diseases from one another through the common use of feeding stations where faeces and food are mixed, and the sharing of small enclosed water sources such as bird baths and ponds (Brittingham *et al.*, 1988). The bacteria most commonly cited as killing house sparrows are *Salmonella enterica* serotype Typhimurium, and *Escherichia coli* subtype 086 (Pennycott *et al.*, 1998). Haemoprotozoa are protozoan (single-celled) eukaryotic organisms that parasitise their host's blood cells for at least one stage of their life-cycle. They are transmitted by vectors such as biting flies. The haemoprotozoan species belonging to the genera *Haemoproteus, Plasmodium and Leucocytozoon* are considered the most likely to be pathogenic to passerine birds (Atkinson and Van Riper, 1991). In contrast, helminth haemoparasites, such as microfilariae, generally are considered non-pathogenic in passerines (Campbell and Ellis, 2007). Coccidian parasites is the general term to identify protozoan parasites belonging to several genera, including *Isospora, Eimeria, and Atoxoplasma*, and passerines tend to be primarily infected by *Isospora* and *Atoxoplasma*, rather than by *Eimeria* (Greiner, 2008).

7.2 AIMS

The aim of the work described in this thesis was to investigate whether or not disease, particularly infectious disease, might play a role in the observed declines in sparrow populations in London. The approach used was to make use of an ongoing RSPB study of sparrow populations at various sites in London, some undergoing population declines and some not, and some with supplementary feeding and some not. In particular, the aims were:

1. To investigate the prevalence, and where possible the intensity, of targeted infectious agents and parasites at the various sites.
2. To investigate markers – in particular haematological markers – of infectious diseases.
3. To investigate variation of infectious agents and parasites across years and seasons, and between age, sex, condition, breeding status, and moult status of infected birds.
4. To compare differences in prevalence and intensity of targeted infectious agents and parasites between: sites that were declining or non-declining, and sites that were provided with supplementary mealworms or not supplemented.
5. To investigate survival of house sparrows in relation to their prevalence and intensity of infectious agents and parasites.

7.3. MAIN FINDINGS

The main findings of this study were:

- The prevalence of *Salmonella enterica* and *Escherichia coli* from bird feeders and bird tables was zero per cent, and only one house sparrow tested positive for *Salmonella enterica* Typhimurium, but none tested positive for *E. coli.*
- The main parasites found belonged to the genus *Plasmodium*, *Atoxoplasma*, and *Isospora*. Prevalence of these parasites were, respectively, 21.8%, 30%, and 10.7%.
- Prevalence of *Atoxoplasma* spp and *Isospora* spp., and intensity of *Plasmodium* spp. and *Atoxoplasma* spp. varied across sites.

- Leucocyte-related values of clinically-healthy house sparrows were generally higher than the normal ranges suggested in reference books for similar-size avian species.
- Birds infected by *Plasmodium* spp., *Atoxoplasma* spp., and *Isospora* spp. showed associated changes in leucocyte- and erythrocyte-related values, indicating that these parasites cause disease.
- Prevalence and intensity of the three parasite genera found (*Plasmodium*, *Atoxoplasma* and *Isospora*) did not vary across the three years of the study, but they showed seasonal variation.
- Adults had higher *Plasmodium* spp. prevalence but lower intensity of infection.
- Females had, in general, higher prevalence of parasites than males.
- Infection with *Plasmodium* spp. was negatively correlated with body condition, especially in juveniles.
- Breeding birds generally had higher prevalence of parasites than non-breeding birds, and higher intensity of *Plasmodium* spp.
- Birds that were moulting generally had higher prevalence and intensity of parasites.
- Birds from declining sites had higher intensity of *Plasmodium* spp. infection, and higher prevalence of *Atoxoplasma* spp. than non-declining sites.
- Birds in fed sites had higher prevalence of *Atoxoplasma* spp. and *Isospora* spp. than unfed sites.
- *Plasmodium* spp. prevalence was correlated with higher survival in autumn, early winter and late winter. Intensity of the parasite was not correlated with survival in autumn or early winter, but it was negatively correlated with survival in late winter.
- Age, sex, and condition were not correlated with survival, when considered alone or interacting with parasites.

7.4. IS DISEASE AN IMPORTANT FACTOR IN THE DECLINE OF THE HOUSE SPARROW IN GREATER LONDON?

In this study it was shown that house sparrows in London are primarily parasitised by three genera of protozoa: the haemoprotozoan *Plasmodium*, and the coccidian *Atoxoplasma* and *Isospora*. Previous studies have reported detrimental effects of these parasites on their host (e.g. Knowles *et al.*, 2010; Hõrak *et al.*, 2004;), including mortality (e.g. Rossi *et* al., 2007; Kozłowski *et al*, 1991; Partington *et al.*, 1989; Var Riper *et al.*, 1986). Of these parasites, those belonging to the genus *Plasmodium* are the most pathogenic (Atkinson and Van Riper, 1991), and birds infected with these parasites suffer high mortality rate (Atkinson *et al.*, 2000; Atkinson *et al.*, 1995).

Parasites of the genera *Plasmodium*, *Atoxoplasma*, and *Isospora* caused disease in house sparrows from the sites considered, as indicated by changes in haematological values above the norm associated with infected individuals.

Parasite prevalence and intensity changed with season and the physiology of the host (Wobeser, 2008). In this study, all three parasites showed a variation between seasons, but not years. There was also a difference in parasite infection between males and females, the latter having higher prevalence than males, although difference in intensity between sexes depended on the parasite genus considered. Several experimental studies have found a link between breeding, and prevalence or intensity of haematozoans (Knowles *et al.*, 2010b; Nordling *et al.*, 1998; Siikamäki *et al.*, 1997; Oppliger *et al.*, 1996; Richner *et al.*, 1995; Norris *et al.*, 1994). Some of these studies found that increased breeding effort can increase parasite prevalence and/ or intensity (meta-analysis in Knowles *et al.*, 2009), presumably through a trade-off between energy allocated to breeding effort and energy allocated to the immune system (Gustaffson *et al.*, 1994).

Other studies have found that parasitism is associated with a cost that is reflected through decreased breeding success (Knowles *et al.*, 2010a; Marzal *et al.*, 2005; Merino *et al.*, 2000). In this study an association was found between breeding and higher parasite prevalence of *Plasmodium* spp.. Breeding is energy-consuming (Gustaffson *et al.*, 1994). Results found in this study suggests, but cannot prove, that there is a trade-off between energy-demanding activities, such as breeding and moulting, and parasite prevalence (and intensity), especially since increased levels of *Plasmodium* spp., a highly pathogenic parasite (Atkinson and Can Riper, 1991), were associated with breeding.

Moulting is another energy-demanding activity (Murphy and King, 1992) that house sparrows have to undergo at least once a year. In this study moulting birds had higher prevalence and intensity of *Plasmodium* spp., further supporting a theory of energetic trade-off between controlling parasite infections and some life-history traits.

The cost of parasitism can also be directly visible through reduced body condition of the host (e.g. Merino *et al.*, 2000). In this study, *Plasmodium* spp. intensity was negatively correlated with body condition. This may mean either that infected birds suffered lower condition as a result of high intensity of parasitism, or that sub-optimal individuals (in lower body condition) were less able to control the infection, especially if they were juveniles.

Nutrients in the diet can affect the immune system (Klasing, 1998), and hence resistance to infectious diseases (Wakelin and Apanius, 1997). Young birds should therefore benefit from supplementary feeding. In particular, nestlings provided with mealworms should be able to control infections, and hence parasite intensity, better than nestlings in unfed sites, because mealworms are rich in proteins which are essential for the immune system (Chandra, 1997). Unfortunately, in this study it was not possible to measure nestling survival or infection status due to logistic constraints. Supplementary feeding did not seem to have an effect on *Plasmodium*

spp. prevalence or infection in juveniles or adults, but it was associated with increased prevalence of gastro-intestinal parasites, probably due to aggregation at mealworm feeders.

To summarise the main points until now, it has been shown that parasites found in house sparrows in this study were pathogenic and should be considered disease, and that they varied with season and were associated with decreased body condition. Furthermore, feeding protein-rich food did not seem to help controlling infections in young birds or adults.

All of these interactions occurred at the individual level, but the impact that parasites might have at the host population level needed to be investigated. Intensity of *Plasmodium*, the most pathogenic of the three genera found in this study (Atkinson and Van Riper, 1991), was higher in declining than in non-declining sites, although prevalence did not vary with population trend. This may indicate that the probability of being infected (prevalence) was the same in declining and non-declining sites, because the probability of becoming infected with the parasite depends mainly on presence or absence of the mosquito vector (Van Riper *et al.*, 1986), while intensity depends on the ability of the host to control the infection (Wakelin and Apanius, 1997). Relapse of infections may be due to seasonal hormonal changes (Valkiunas, *et al.*, 2004; Applegate, 1970; Applegate and Beaudoin, 1970), or stress factors (Navarro *et al.*, 2004; Valkiūnas *et al.*, 2004). In declining sites, there may be some unidentified factors associated with the individual birds which make them less able to control the infection.

House sparrows in declining sites had higher intensity of the highly pathogenic *Plasmodium* spp., but a link between parasites and reduced survival was needed. Investigation of overwinter survival in this study showed a negative correlation with *Plasmodium* intensity in late winter. The strength of the relationship between survival and parasite prevalence and intensity changed as winter progressed. In autumn, prevalence but not intensity, of the parasite was higher in birds that were re-

sighted. The same result was found at the beginning of winter, probably because birds re-sighted had very low and/or chronic infections, especially in autumn when the sample of re-sighted individuals was adult-biased, and adults have higher prevalence of parasitism than juveniles (shown in chapter 4). However, adults may be more able to control the infection once it has been acquired, because they have already been selected for survival and developed acquired immunity. Ability to control infections can be altered by environmental changes (Acevedo-Whitehouse and Duffus, 2009; Wobeser, 2008). This is supported by the finding that in late winter, when weather condition are worse, intensity of *Plasmodium* spp. infection was negatively correlated with probability of survival. The result was consistent even when only birds that were re-sighted in autumn were considered for analyses in late winter. This was done in order to control for the bias towards decreased re-sighting probability created by dispersal or movements.

Is disease an important factor in the decline of the house sparrow in Greater London? This study showed that parasites found were causing disease, and that they were associated with population decline and reduced survival. However, a causal link cannot be established, especially since some infections may be subclinical at the moment of sampling, but they may become apparent and influence productivity or survival of the host later on (Wobeser, 2008). Productivity data would also be needed to further investigate the relationship between parasites and breeding success in house sparrows in London. This could be the aim of further studies, which should also investigate the effect of *Plasmodium* spp. on non-declining species sympatric to the house sparrow, to understand if any aspects of the biology and ecology of this passerine may affect its ability, if so, to fight infections.

Species of garden birds such as blue tits, great tits, finches and dunnocks could be sampled for the same parasites identified in this study. The hypotheses would be that a) the gregarious nature of house sparrows makes them more prone to pathogens, either through contact with other diseased individuals or social stress; b) house sparrows have longer breeding seasons because they are multi-brooded, and this may

add to their energy demand, and increase their 'stress' of breeding, and they would be less able to fight and control infections. In order to test for the first hypothesis, other gregarious garden species, such as finches and starlings could be sampled, as they also feed on the ground as well as on bird feeders, together with non-gregarious species such as tits, and ground-feeding non-gregarious species such as dunnocks. If gregarious species had higher prevalence and especially intensity of pathogens than non-gregarious species, the next step would be to identify whether other aspects (distance between nest-holes and number of feeding station) may be linked to the problem, and mitigation measurements could be used, such as providing more feeding stations to avoid competition at feeding stations, or providing more nest holes. The second hypothesis could be tested in the field by monitoring adults on the nest. This would be almost impossible in the current set up, due to the inaccessibility of nest sites, and the risk of desertion of adults which may occur throughout the breeding season, but especially at egg-laying stage (Summers-Smith, *pers. comm.*). An alternative test for the second hypothesis is to control the breeding cycle of birds experimentally, to initiate, halt and prolongue the breeding cycle at different stages to test whether that may alter the prevalence and intensity of haemoparasites. This would require taking birds in captivity, which may introduce further biases such as altering of their normal behaviour or increase stress levels due to the captive state.

Pathogenic agents rarely act in isolation to impact the fitness and survival of their host (Acevedo-Whitehouse and Duffus, 2009). More often, diseases act synergistically with other factors, such as predation, food scarcity, competition with other species, and other stress factors to cause a detrimental effect in their host. Several causes for the decline of the house sparrow have been investigated and tested in the past decade, but none have proved to be the definitive answer to explain the decline, or reverse it. The decline of the house sparrow in London is probably due to the synergistic effect of several factors, of which disease is likely to be an important one.

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